Neuromodulation with Transcranial Direct Current Stimulation
The Influence of Electrode Arrangement

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Neuromodulation with Transcranial Direct Current Stimulation: The Influence of Electrode Arrangement

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Abstract

Transcranial direct current stimulation (tDCS) could improve plasticity and motor function, but the influence of electrode arrangement is unclear. The aim of this PhD was to develop and utilise a sequential learning paradigm involving gross movements of the hand to assess the effect of tDCS electrode arrangement on; i) motor sequence learning in healthy young and older adults, ii) motor sequence learning and upper limb function in chronic stroke survivors and iii) retention of learning in healthy adults, and to determine whether the response to tDCS is dependent on changes in transcallosal inhibition (TCI).

Study one tested the motor sequence learning paradigm. Young adults, stroke survivors and age-matched controls all demonstrated improvements in motor preparation with 25 repetitions of a movement sequence. However, stroke survivors showed impaired sequence specific learning. Study two demonstrated that healthy ageing was associated with reduced motor sequence learning, but tDCS did not affect performance for either younger or older adults. Bihemispheric tDCS led to an increase in TCI (ipsilateral silent period duration) for the younger group only. There were no significant relationships between changes in TCI and learning. Study three demonstrated a significant effect of tDCS electrode arrangement on upper limb function in stroke survivors, with improvements after unilateral tDCS (anodal or cathodal), but not after bihemispheric. However, there was no effect of tDCS on motor sequence learning or the change in TCI from either hemisphere. Study four showed no effect of tDCS on 48 hour retention of learning for healthy adults. However, cathodal tDCS delivered during training impaired later re-learning of the movement sequence.

The findings of these studies suggest that tDCS does not improve learning of a sequence of gross hand movements. High variability in response is observed and there is no consistent effect of tDCS on TCI.

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Communications arising from this PhD

Peer reviewed publications

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Fleming MK, Newham DJ and Rothwell JC (In press). Explicit motor sequence learning with the paretic arm after stroke. *Disability and Rehabilitation.*

Fleming MK and Newham DJ (2017). Reliability of Transcallosal Inhibition in Healthy Adults. *Frontiers in Human Neuroscience 10:681.*

In Review

Fleming MK, Rothwell JC, Sztriha L, Teo JT and Newham DJ (In review). The effect of transcranial direct current stimulation on motor sequence learning and upper limb function after stroke. *Clinical Neurophysiology.*

Conference abstracts: Oral Presentations

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Fleming MK (2016). The effect of transcranial direct current stimulation on motor sequence learning and upper limb function after stroke. *King's college London postgraduate symposium. (Award "best divisional oral presentation")*

Fleming MK, Lazarus NR and Newham DJ (2016). Does age affect motor sequence learning ability? *Muscles and Movement: A Roger Woledge memorial symposium, London, UK.*

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Conference Abstracts: Poster presentations

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Abbreviations

Abbreviation	Meaning
AgCl	Silver Chloride
ARAT	Action Research Arm Test
AMT	Active motor threshold
ANOVA	Analysis of variance
Arb.	Arbitrary
AUC	Area under the curve
CIMT	Constraint induced movement therapy
CST	Corticospinal tract
DTI	Diffusion tensor imaging
EMG	Electromyography
ES	Effect size
FA	Fractional anisotropy
FDI	First dorsal interosseous
FM	Fugl-meyer assessment
FMRI	Functional magnetic resonance imaging
GABA	γ-Aminobutyric acid
H	Haemorrhagic
I	Ischemic
IHI	Interhemispheric inhibition
iSP	Ipsilateral silent period
JTT	Jebsen Taylor test
LTP	Long term potentiation
M1	Primary motor cortex
MCA	Middle cerebral artery
MEP	Motor evoked potential
mo	Months
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MSO	Maximum stimulator output
MT	Movement time
n	Number of participants

NaCl	Sodium Chloride
NIHSS	National institute for health stroke scale
NMDA	N-methyl-D-aspartate
OMCASS	Orgogozo MCA scale
OT	Onset Time
PI	Performance Index
PL	Path length
PMC	Premotor cortex
PMd	Dorsal premotor cortex
rmANOVA	Repeated measures analysis of variance
RMS	Root mean square
RMT	Resting motor threshold
RT	Reaction time
rTMS	Repetitive transcranial magnetic stimulation
S	Session
SD	Standard deviation
SEM	Standard error of the mean
SICI	Short-latency intracortical inhibition
SMA	Supplementary motor area
SPL	Superior parietal lobe
SRTT	Serial reaction time task
SVIPT	Sequential visual isometric pinch task
TCI	Transcallosal inhibition
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation

Chapter 1 Introduction

The concept of applying electricity to alter the activity of the human brain has been studied since ancient times (Sarmiento et al., 2016). Yet, it is only in the past few decades that the scientific and clinical community has shown widespread interest in the potential for transcranial direct current stimulation (tDCS) to modulate cortical excitability and affect motor function and cognition. As a painless and relatively inexpensive technique, tDCS is an ideal brain stimulation method for double-blind sham controlled studies in combination with cognitive or motor tasks.

Low intensity, constant electric current (usually 1 - 2 mA) is applied to the cortex via carbon electrodes encased in saline-soaked sponges placed directly on the scalp and secured with elastic headbands. There are a number of parameters to consider for tDCS studies, including: 1) the duration of stimulation; 2) the intensity of stimulation; 3) the electrode size; and 4) the electrode arrangement. The primary aim of this thesis was to examine the effect of tDCS electrode arrangement on motor sequence learning, motor control and cortical activity in healthy adults and stroke survivors with upper limb impairment.

In Chapter 2 the previous literature that informed the studies for this thesis is reviewed. In particular, an overview of the physiology that underlies the effects of tDCS on the motor system, studies applying tDCS to the primary motor cortex (M1) of healthy adults and stroke survivors with the intention of assessing changes in cortical activity or motor performance, and the effects of tDCS on motor learning.

Chapter 3 presents the experiments to pilot the computer based motor sequence learning paradigm. Experiment one assesses whether healthy adults demonstrate learning over multiple repetitions of a movement sequence, in a similar manner to that seen with other sequence learning paradigms. Experiment two tests whether the task can be used in a repeated measures

study design by assessing learning with weekly exposure. Finally, experiment three investigates whether stroke survivors with upper limb impairment are capable of performing the task with their paretic arm and whether they demonstrate similar motor sequence learning as healthy, age-matched controls.

Chapters 4 through 6 report studies testing the effect of the common tDCS electrode arrangements on motor sequence learning in healthy ageing (Chapter 4) and stroke survivors with upper limb impairment (Chapter 5). Based on the interhemispheric competition model (Murase et al., 2004; Nowak et al., 2009), the active tDCS conditions included are: i) anodal to attempt to increase excitability of M1 contralateral to the hand performing the task (right M1 for healthy adults, ipsilesional M1 for stroke survivors); ii) cathodal to attempt to decrease excitability of M1 ipsilateral to the hand performing the task (left M1 for healthy adults, contralesional M1 for stroke survivors); and iii) bihemispheric (anodal and cathodal concurrently). In each study, the change in transcallosal inhibition is assessed using transcranial magnetic stimulation to attempt to probe for a potential mechanism underlying changes.

In Chapters 4 and 5, online performance of the motor sequence learning task is assessed. Using a within-subjects design, participants receive each of the tDCS electrode arrangements in a randomised order in addition to a sham stimulation session. For the stroke survivors, upper limb function is also assessed using the Jebsen Taylor hand function test (Jebsen et al., 1969) to systematically assess the effect of electrode arrangement on function of the paretic arm.

Finally, Chapter 6 tests the effect of tDCS electrode arrangement on the retention of motor sequence learning. Using a between-subjects study design healthy adults are randomised to receive one of the electrode arrangements during performance of the task. Retention of learning-related improvements and re-learning of the movement sequence are assessed 48 hours later.

Chapter 2 Review of Literature

In the review of literature, the effect of transcranial direct current stimulation (tDCS) on the motor system will be discussed with application to motor learning and upper limb function. The scope of this review is limited to studies with human subjects targeting the motor cortex for the assessment of changes in the control of the upper limb. In the first section, the likely mechanisms of action will be outlined and the factors that can be modified to alter the effect of tDCS will be discussed. In the second section, the concept of motor learning will be introduced and studies that have delivered tDCS to alter motor learning in healthy adults and stroke survivors will be presented. Finally, in the third section of this review, the effect of single and multiple sessions of tDCS on upper limb function in healthy adults and stroke survivors will be summarised and critiqued.

2.1 Neuromodulation with transcranial direct current stimulation

2.1.1 General information

Transcranial direct current stimulation is a safe, painless and non-invasive method for stimulating the human brain. Low intensity, direct, constant current is applied to the scalp and corticospinal excitability can increase or decrease depending on the stimulation parameters (Nitsche and Paulus, 2000). When applied to the primary motor cortex (M1), anodal tDCS increases, whereas cathodal tDCS typically decreases, corticospinal excitability assessed using transcranial magnetic stimulation (TMS; Nitsche and Paulus, 2000). Although the exact mechanism of action is not completely understood (Roche et al., 2015), the effects are thought to be due to modulation of cortical neuron excitability (Nitsche et al., 2003a; Nitsche et al., 2003b) through a shift in membrane potential, and effects are dependent on the activity of a number of neurotransmitters and membrane channels (Medeiros et al., 2012). Pharmacological studies indicate that the effects of tDCS on cortical excitability are influenced by drugs which

modify membrane potential, synaptic plasticity and N-methyl-D-aspartate (NMDA) receptor activity, and studies with paired pulse TMS and magnetic resonance spectroscopy (MRS) suggest modulation of inhibitory circuits, including γ-Aminobutyric acid (GABA) concentration (Kidgell et al., 2013a; Medeiros et al., 2012; Nitsche et al., 2003a; Stagg et al., 2009).

There are a number of factors which could affect the electric field distribution with tDCS, including anatomical differences between people, the properties of the underlying tissues and the size and arrangement of the electrodes. Typically "unilateral" motor cortex stimulation involves the placement of one electrode over the M1 and the other on the contralateral supraorbital ridge. "Bihemispheric" stimulation is when one electrode is placed on each M1 (also referred to as "dual", "bilateral" or "M1-M1"). The electric field strength tends to be strongest close to the anode and therefore differing electrode arrangements can lead to different patterns of current spread within the cortex (Moliadze et al., 2010; Opitz et al., 2015). Resting state functional magnetic resonance imaging (FMRI) indicates different cortical network changes during bihemispheric compared with unilateral tDCS (Lindenberg et al., 2016; Sehm et al., 2012), although the exact pattern of differences across electrode arrangements is not yet clear.

The hypothesis that bihemispheric tDCS could provide additional benefit over unilateral stimulation stems from the theory that tDCS can exert effects locally but also through modulation of transcallosal inhibition. In stroke survivors it is theorised that there are abnormal levels of interhemispheric inhibition from the contralesional to the ipsilesional M1, resulting in an imbalance in relative levels of cortical excitability which correlates with severity of functional impairment (Murase et al., 2004; Nowak et al., 2009; Takeuchi et al., 2010; Takeuchi and Izumi, 2012). As a result, three main strategies for delivering tDCS in stroke are typically investigated:

1) Facilitation of ipsilesional M1 activity with anodal tDCS
2) Suppression of contralesional M1 activity with cathodal tDCS (thereby reducing the abnormal inhibitory drive toward the lesioned hemisphere)

3) Facilitation of ipsilesional M1 and suppression of contralesional M1 simultaneously with bihemispheric tDCS.

A similar approach is often taken with healthy adults; anodal tDCS to target the cortex contralateral to the performing hand, cathodal to target the ipsilateral cortex and reduce transcallosal inhibition or bihemispheric stimulation. However, the hemisphere that is the target for facilitation differs across studies and the impact of electrode arrangement is not well understood.

2.1.2 The influence of tDCS polarity on change in M1 activity

Changes in corticospinal excitability are evident both during and after stimulation. Nitsche and Paulus (2000) initially demonstrated that 4 s of 1 mA anodal tDCS increased motor evoked potential (MEP) amplitude and cathodal decreased it, indicating a rapid change in corticospinal excitability. When tDCS was delivered for five minutes, the MEP changes persisted for four minutes with anodal stimulation and three minutes following cathodal. The duration of the excitability change was dependent on the intensity and duration of stimulation, with at least 0.6 mA and at least three minutes required to achieve after-effects (Nitsche and Paulus, 2000). In a follow-up study, Nitsche et al. (2003b) further investigated the effect of cathodal tDCS, finding that long-lasting (> 60 minutes) decreases in excitability could be induced following just 9 minutes of stimulation. Responses to transcranial electrical stimulation and H reflexes were unchanged, suggesting that the effect of tDCS was due to modulation of cortical neurons rather than spinal motoneurons. Changes in MEP amplitude have also been demonstrated in other studies (Ardolino et al., 2005; Bastani and Jaberzadeh, 2012; Di Lazzaro et al., 2012; Kidgell et al., 2013a; Lang et al., 2004; Moliadze et al., 2014), without a change in resting motor threshold (RMT; Batsikadze et al., 2013; Di Lazzaro et al., 2012; Nitsche et al., 2005). This further suggests that tDCS is primarily targeting cortical interneurons rather than the pyramidal tract neurons directly (Stagg and Nitsche, 2011).

Although there are three commonly used electrode arrangements (anodal, cathodal and bihemispheric) the influence of the arrangement on modulation of MEP amplitude is unclear. Kidgell et al. (2013b) delivered 13 minutes of unilateral anodal (over right M1), bihemispheric (anode over right M1, cathode over left M1), or sham stimulation and induced MEP facilitation from the left extensor carpi radialis longus that was similar in magnitude across unilateral and bihemispheric conditions but not for sham. Similarly, with older adults, the same group of authors (Goodwill et al., 2013) showed an increase in excitability of the non-dominant M1 following both anodal and bihemispheric tDCS but no significant difference between them. These results combined suggest that bihemispheric stimulation may not induce any additional modulation of excitability of the target cortex over unilateral. O'Shea et al. (2014) also found that the effects of bihemispheric tDCS were not simply a sum of anodal and cathodal effects. In their study, 20 minutes of anodal tDCS to left M1 increased the amplitude of MEPs from both hemispheres suggesting a global increase in excitability. Cathodal tDCS to right M1 decreased MEP amplitude from the right M1 and also increased MEP amplitude from the left M1, consistent with the concept of reducing cortical excitability with cathodal stimulation in order to increase excitability of the opposite hemisphere via reductions in transcallosal inhibition. In contrast, bihemipheric tDCS failed to produce significant changes in excitability for either hemisphere. However, the authors also reported that the response to anodal and cathodal stimulation could predict the response to bihemispheric stimulation, suggesting that there was some change in excitability following bihemispheric tDCS which was variable across participants. They found that when a participant showed a greater increase in left M1 excitability with anodal stimulation, they had less of a decrease in right M1 excitability with cathodal tDCS, suggesting that some participants may be more susceptible to facilitation over suppression or *vice versa*.

The effect of electrode arrangement on movement induced cortical activation has been studied in healthy older adults (61 – 77 years) using FMRI (Lindenberg et al., 2013). Anodal (left M1) and bihemispheric tDCS were delivered for 30 minutes and cortical activation assessed during a

choice reaction time tapping task. They found differences in M1 activation between bihemispheric and anodal stimulation that depended on which hand was moving. When the right hand was performing the task there was greater activation in the ipsilateral M1 during bihemispheric stimulation compared with anodal. However, neither active condition was significantly different to sham. There were no differences in activation for the left M1 which was the site of the anode in both active conditions. When the left hand was performing the task there was a bilateral increase in M1 activity during the bihemispheric tDCS condition compared with anodal, but once again no differences compared with sham. This indicates that changes in motor cortex activity with bihemispheric tDCS cannot be simply explained as facilitation of the motor cortex under the anode and suppression of activity under the cathode. This appears consistent with findings using TMS (O'Shea et al., 2014) described previously (page 22). Lindenberg et al. (2013) also reported a correlation between fractional anisotropy (FA) values of transcallosal connections (as measured by diffusion tensor imaging (DTI)) and changes in the laterality of activation for the left tap condition, but not the right tap. The authors suggested that this means that the integrity of transcallosal connections plays a role in the pattern of tDCS induced changes in cortical activation, and argued that their finding is consistent with the hypothesis that the effects of tDCS on the M1 opposite the anode are mediated by transcallosal fibres. However, they did not test tDCS with the anode over the right M1 so it is unknown whether the pattern of activation changes depends on hemispheric dominance.

2.1.3 Pharmacological studies

Pharmacological studies indicate alteration in tDCS effects when consuming drugs that are known to influence sodium or calcium channels, or NMDA receptor activity (Liebetanz et al., 2002; Nitsche et al., 2003a). Blocking voltage-dependent sodium channels with Carbamazepine or calcium channels with Flunarizine prevented or reduced the enhancement of excitability with anodal tDCS, but had no effect on cathodal tDCS. Using the NMDA receptor antagonist Dextromethorphane had no effect on changes in excitability during a short period of stimulation,

but prevented long-lasting increases (anodal) or decreases (cathodal) in excitability (Liebetanz et al., 2002; Nitsche et al., 2003a). These results provide evidence that the modulation of excitability with anodal tDCS is dependent on depolarisation of membrane potential. Cathodal effects appear to be unchanged with Carbamazepine, suggesting that cathodal tDCS leads to hyperpolarisation of membrane potential which is not affected by this drug. Dextromethorphan blocks the after-effects of both anodal and cathodal tDCS, suggesting that the long lasting effects of tDCS are due to synaptic plasticity and are reliant on NMDA receptor activity.

2.1.4 Change in inhibition

Inhibitory circuits are also shown to be affected by tDCS. Using MRS, a reduction in GABA concentration has been observed with anodal tDCS (Kim et al., 2014; Stagg et al., 2009) which correlates with motor adaptation (Kim et al., 2014). Interestingly, Stagg et al. (2009) found a reduction in both GABA and glutamate with cathodal tDCS. The authors speculated that the reductions in GABA were due to decreased activity of glutamic acid decarboxylase 67 and the reduction in glutamate resulted from reduced synthesis from glutamine.

However, the effect of tDCS on short latency intracortical inhibition (SICI) assessed using paired pulse TMS is less clear. Di Lazzaro et al. (2012) found no changes in SICI with cathodal tDCS to right M1 whereas others (Kidgell et al., 2013a; Nitsche et al., 2005) have found a significant reduction in SICI following anodal tDCS and an increase with cathodal tDCS to left M1. Variations in stimulation parameters could account for these differing results. Di Lazzaro et al. (2012) assessed SICI using just five stimuli which may not have been sufficient to produce reliable results. Additionally, they delivered tDCS to the right M1 rather than the left. Finally, Di Lazzaro et al. (2012) delivered tDCS for 20 minutes, whereas Nitsche et al. (2005) used 7 minutes. Increasing the duration of stimulation does not necessarily enhance or prolong the effects. Monte-Silva et al. (2013) demonstrated that although 13 minutes of anodal tDCS increased MEP amplitude, doubling the duration led to a decrease in MEP amplitude instead, which is thought

to be due to an intrinsic neuronal mechanism which prevents over-excitation. It is possible that a similar mechanism exists for modulation of GABAergic interneurons and that the increase in SICI seen with a short duration of tDCS is reversed if stimulation is prolonged. This hypothesis is consistent with the results of Tremblay et al. (2013) who found that 20 minutes of cathodal tDCS did not modulate either cortical silent period duration or long interval intracortical inhibition, which are thought to be mediated by $GABA_B$ receptors. However, with anodal tDCS they did find an increase in MEP amplitude and shortened duration of the cortical silent period. Combined, these results indicate a complex modulation of GABA mediated inhibition within the motor cortex.

Few studies have assessed the modulation of transcallosal inhibition (TCI) with tDCS even though balancing of TCI is frequently cited as a rationale for delivering either cathodal or bihemispheric tDCS. The studies that have tested this measure, using the double pulse (dual coil) method, have lent support to this rationale. Tazoe et al. (2014) assessed the effect of tDCS electrode arrangement with 15 minutes of 1.5 mA stimulation; anodal to right M1, cathodal to left M1 or bihemispheric. For anodal stimulation they found an increase in right to left interhemispheric inhibition (IHI), but no changes for left to right IHI. For cathodal stimulation there was a decrease in IHI from both hemispheres. For bihemispheric stimulation there was an increase in IHI from right to left M1 as well as a decrease from left to right M1 (for diagram of changes see Figure 2.1). These results were consistent with the authors' hypothesis, with IHI increasing from the hemisphere where neuronal excitability was increased (anodal and bihemispheric), and decreasing from the hemisphere where excitability was decreased (cathodal and bihemispheric). Decreases in IHI have also been observed in stroke survivors from contralesional to ipsilesional M1 after 10 days of combined bihemispheric tDCS and constraint induced movement therapy (Bolognini et al., 2011). These results suggest that tDCS also modulates excitability of transcallosal neurons.

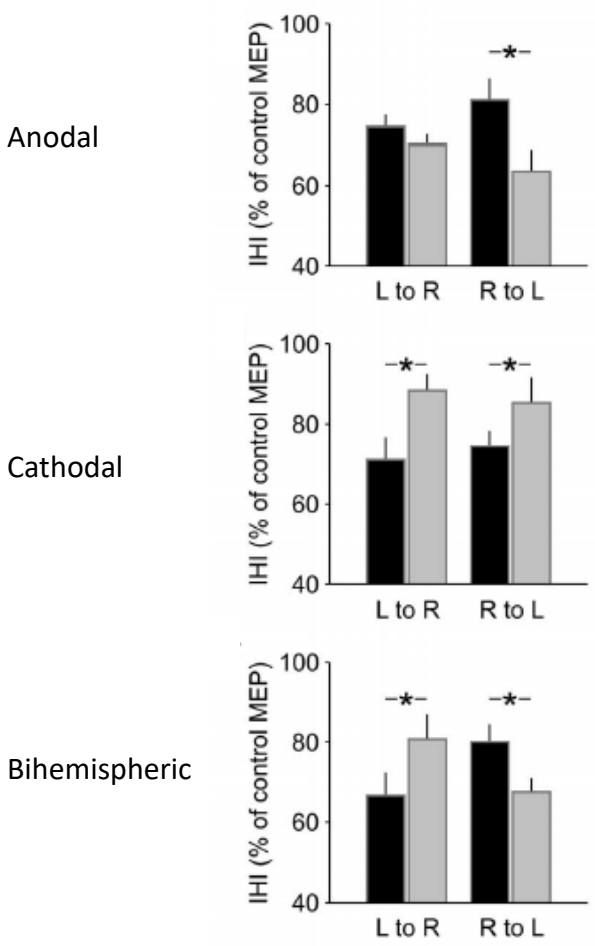


Figure 2.1 Diagram showing results of changes in IHI following 15 minutes of 1.5 mA tDCS.
Black bars = pre, Grey bars = post stimulation. Adapted from Tazoe et al. (2014) Figure 1, page 5.

However, there are two methods of assessing inhibition between cortices using TMS and Lang et al. (2004) found no change in ipsilateral silent period (iSP) duration from the left hand (left-right M1 TCI) following either anodal or cathodal tDCS delivered to the left M1 of healthy young adults. There was, however, a significant transient increase in iSP duration from the right hand (right-left M1 TCI) with anodal stimulation, and a decrease with cathodal. This is perhaps surprising, given that the tDCS was applied to the left M1, but indicates a complex modulation of interhemispheric connections. The analysis of inhibition between hemispheres using double pulse (dual coil) TMS compared with the iSP may reflect different mechanisms (Chen et al., 2003) as the dual coil method measures the reduction in MEP amplitude with conditioning of the opposite M1 whereas the iSP method measures the disruption of voluntary muscle activity.

Further research is required to fully understand the effects of tDCS on TCI and how changes might relate to motor function.

2.1.5 The effect of changing current intensity and electrode size

The influence of current intensity on the change in corticospinal excitability has been investigated by several studies with varying stimulation parameters. Kidgell et al. (2013a) compared changes in MEP amplitude during weak voluntary contraction following 10 minutes of 0.8, 1.0 and 1.2 mA anodal tDCS (25 cm^2 electrode) in a crossover design and found no significant differences between stimulation intensities. The authors therefore concluded that different stimulation intensities did not differentially modulate corticospinal excitability. However, this is a very narrow range of stimulation intensities and they chose to measure MEP amplitude only at 120 % active motor threshold (AMT) rather than assessing excitability over the range of a stimulus response curve which may have been more sensitive. In contrast, Bastani and Jaberzadeh (2013a) demonstrated differences in MEP facilitation between 0.3, 0.7, 1.4 and 2 mA anodal tDCS (24 cm^2 anode). There was a greater facilitation at 2 mA than 1.4 mA suggesting that a stronger current has a greater modulatory effect. Interestingly, they found greater MEP facilitation with 0.3 mA than 0.7 mA, indicating that further investigation is required to elucidate the effects of lower current intensities. However, Batsikadze et al. (2013) found that cathodal tDCS delivered for 20 minutes at 2 mA (35 cm^2 electrode) led to an increase in corticospinal excitability instead of the expected decrease. Anodal tDCS still gave the expected increase in excitability, and cathodal tDCS at 1 mA did show a significant reduction in excitability. The reason underlying the non-linear effects of intensity are currently unknown, but Batsikadze et al. speculated that it could be due to increases in calcium influx leading to long term potentiation, rather than long term depression, increased depolarisation of dendrites or stimulation of other brain regions. Combined these results indicate that the effects of current intensity are complex, particularly when attempting to suppress cortical excitability.

The size of the electrodes also has an influence. Bastani and Jaberzadeh (2013b) used three different electrode sizes and altered the current intensity so that the current density was kept consistent, as current density is a factor affecting the magnitude of corticospinal changes (Nitsche and Paulus, 2000). The increase in corticospinal excitability following 10 minutes of anodal stimulation was significantly greater for the 12 cm^2 electrode than for the 24 or 35 cm^2 but did not differ between the 24 and 35 cm^2 electrodes. The excitability changes persisted for at least 30 minutes with the 12 and 24 cm^2 electrodes, but only up to 10 minutes following stimulation with the 35 cm^2 electrode. The authors speculated that the reason for the differences between electrode sizes was due to the spread of current; that the larger and less focal electrodes resulted in stimulation of areas adjacent to M1 that may have had inhibitory effects.

2.1.6 Other factors affecting response to tDCS

Ageing may alter the time-course of changes in MEP amplitude with tDCS. Fujiyama et al. (2014) showed that young adults (mean 23 years) had a rapid increase in MEP amplitude, which was significantly different to sham immediately and 10 minutes following anodal stimulation, whereas older adults (mean 68 years) showed no difference initially but an increase in MEP amplitude at 20 and 30 minutes following stimulation. Similarly, Goh et al. (2015) found an increase in MEP amplitude following anodal tDCS to the ipsilesional M1 of chronic stroke survivors (mean age 60 years) that reached significance after 30 minutes and persisted for at least 60 minutes. This indicates that the plastic response to tDCS might be delayed in older adults which could have implications for the timing of delivery of tDCS when combined with motor training in that population.

Kim and Ko (2013) examined the effect of voluntary exercise on changes in MEP amplitude of right first dorsal interroseus (FDI). There were four groups which received anodal or sham tDCS with or without a 30 s bout of a voluntary grip exercise at the end of the stimulation period.

There was a significant increase in MEP amplitude with active tDCS which was greatest for the group with exercise, but there was no difference between the group that received tDCS alone and the group who performed the exercise alone (sham tDCS). Therefore, combining tDCS with exercise resulted in improved facilitation of corticospinal excitability, but the changes with tDCS alone were comparable to those with exercise alone. This has implications for the use of tDCS as an adjuvant to physical rehabilitation, potentially suggesting that tDCS should be applied alongside motor practice for maximum effect. However, these effects were on corticospinal excitability, rather than motor function specifically, and the exercise was only at the very end of the 20 minute stimulation period.

The effect of repeated applications of tDCS on changes in M1 excitability is dependent on the relative timing of the stimulation periods. Studies have demonstrated that doubling the stimulation time from 5 minutes to 10 (Fricke et al., 2011), or from 9 to 18 minutes (Monte-Silva et al., 2010) prolongs the duration of the effect on MEP amplitude. However, Fricke et al. (2011) found that if breaks of 3 or 10 minutes were introduced between the 5 minute periods then the direction of modulation was reversed, likely due to homeostatic mechanisms which regulate neuroplasticity based on previous activity. Similar results were found when two consecutive 13 minute periods of anodal tDCS were delivered targeting abductor digiti minimi (Monte-Silva et al., 2013). These findings highlight the fact that increasing the duration of stimulation does not simply prolong its effects and that the relative timing of stimulation periods should be considered carefully if multiple bouts are to be delivered.

2.2 Motor learning

2.2.1 General

Motor learning involves the refinement of factors such as the relative timing of muscle activations, changes in the positions of the necessary effectors and knowledge of the sequence of required movement trajectories specific to the trained movement or skill. Practice-dependent improvements in motor performance result, which persist beyond the period of training. A shift in the speed-accuracy trade-off may be required, which indicates that the motor system has become capable of producing quicker movements without sacrificing accuracy (Dayan and Cohen, 2011; Lefebvre et al., 2012a; Reis et al., 2008; Reis et al., 2009). Motor learning involves long term potentiation (LTP)-like processes (Dayan and Cohen, 2011; Rioult-Pedotti et al., 2000; Stefan et al., 2006; Ziemann et al., 2004), M1 plasticity during motor training is dependent on learning (Kleim et al., 1998) and increases in M1 grey matter volume are seen with performance improvements after a period of training (Gryga et al., 2012; Sampaio-Baptista et al., 2014).

It has been suggested that the initial and later phases of motor skill learning are distinct and mediated by different networks of brain regions (Hikosaka et al., 2002; Karni et al., 1998). The initial rapid learning reflects the acquisition of a task-specific processing routine and involves prefrontal, parietal, premotor (PMC), M1 and supplementary motor area (SMA), whereas the later and more gradual improvements in performance involve ongoing modifications of links between motor regions of the basal ganglia, the cerebellum and M1. The duration of each learning phase depends on task complexity. Motor learning is frequently assessed experimentally using sequential finger movement paradigms such as the serial reaction time task (SRTT) which relate to motor skills such as writing, typing or playing musical instruments, or by sensorimotor tasks such as the sequential visual isometric pinch task (SVIPT) whereby modification of force is required to follow a pattern of changes in visual targets. These tasks can be “implicit” where the participant is not told of the presence of the underlying sequence but

changes in performance still occur, or "explicit" where the participant is told of the sequence and can attend to it and deliberately try to learn the pattern of movements. Hardwick et al. (2013) combined data from FMRI studies to explore the brain regions representing "core motor learning", i.e. regions involved in both sequence and sensorimotor learning tasks. When grouped together these learning tasks consistently activated dorsal premotor cortex (PMd), M1, SMA and lobule VI of the cerebellum. When the task types were directly compared, the SRTT produced more activation of PMC, SMA, superior parietal lobe (SPL) and thalamus whereas sensorimotor tasks showed greater cerebellum and basal ganglia activation. Explicit SRTT variants showed stronger PMd, SMA, SPL and thalamus activation than implicit SRTT variants (Hardwick et al., 2013).

There are multiple stages of motor skill learning; 1) practice effects resulting in immediate improvement in performance (online learning), 2) memory consolidation that occurs between sessions (offline effects) and 3) long-term memory formation resulting in successful retention after days or longer (Censor et al., 2012; Dayan and Cohen, 2011; Robertson et al., 2004). Throughout this thesis, the term "motor sequence learning" refers to the online learning process and "retention" refers to the consolidation of motor learning tested days or weeks later, unless otherwise stated.

2.2.2 The influence of tDCS on motor learning in healthy adults

The effect of tDCS on motor learning is inconsistent across studies, with differing tasks, stimulation parameters and study designs. Some have tested the dominant hand (Kang and Paik, 2011; Stagg et al., 2011; Zimerman et al., 2013), whereas others have chosen to target the non-dominant hand (Kantak et al., 2012; Karok and Witney, 2013; Rroji et al., 2015; Vines et al., 2008). Unless otherwise specified, studies reviewed in this section have delivered anodal tDCS to the M1 contralateral to the performing hand, cathodal to M1 ipsilateral to the performing hand or bihemispheric (both simultaneously).

Improvements in implicit (Kantak et al., 2012; Nitsche et al., 2003c) and explicit (Karok and Witney, 2013; Stagg et al., 2011) motor sequence learning have been demonstrated with active tDCS compared to sham. Stagg et al. (2011) further demonstrated that changes in performance were polarity and timing specific as cathodal tDCS delivered to the M1 *contralateral* to the hand performing the task, or anodal tDCS delivered *prior* to the task, instead of during, led to impaired learning. In contrast, Kang and Paik (2011) found no differences in the specificity of implicit sequence learning (SRTT) between anodal, bihemispheric and sham stimulation when tested immediately or 24 hours following the training of the movement sequence. However, the authors did not report whether the rate of improvement in reaction times during training differed between stimulation types or whether any participants noticed the presence of a repeating sequence which could affect the "implicit" nature of the task. Perruchet et al. (1997) found that participants were able to recognise components of a repeated sequence for an implicit learning task and Howard et al. (2004) reported that most participants actively search for a pattern. Given that the study by Kang and Paik was a cross-over design, if participants had noticed a repeating sequence then their performance in subsequent sessions may have been altered as a result.

Karok and Witney (2013) examined changes in M1 excitability, in addition to testing explicit sequence learning with the non-dominant (left) hand. They reported significant improvements in learning with bihemispheric tDCS compared to sham. There was also a tendency for anodal tDCS at the first post-test, but no differences at the other time points, which might suggest greater efficacy of bihemispheric tDCS over anodal for explicit learning, consistent with the findings of Vines et al. (2008). The changes in the excitability of the right hemisphere (site of the anode) were as expected; increases in excitability with both active tDCS conditions, but not sham. Bihemispheric tDCS led to a significantly greater magnitude of MEP facilitation than anodal. For the left M1 (site of the cathode), bihemispheric tDCS led to a decrease in excitability but anodal and sham stimulation did not (Karok and Witney, 2013). These results contrast with those of

O'Shea et al. (2014) who did not find a significant change in MEP amplitude of either hemisphere following bihemispheric tDCS but high between-subject variability. The differences, represented in Figure 2.2, could lie in the targeting of the right (Karok and Witney, 2013) *vs* left (O'Shea et al., 2014) M1, and in the activity of the left hand to perform the motor task during tDCS for Karok and Witney which may have enhanced the cortical effects. Additionally, Karok and Witney had smaller electrodes than O'Shea et al. and a larger sample size (n = 20 *vs* 13).

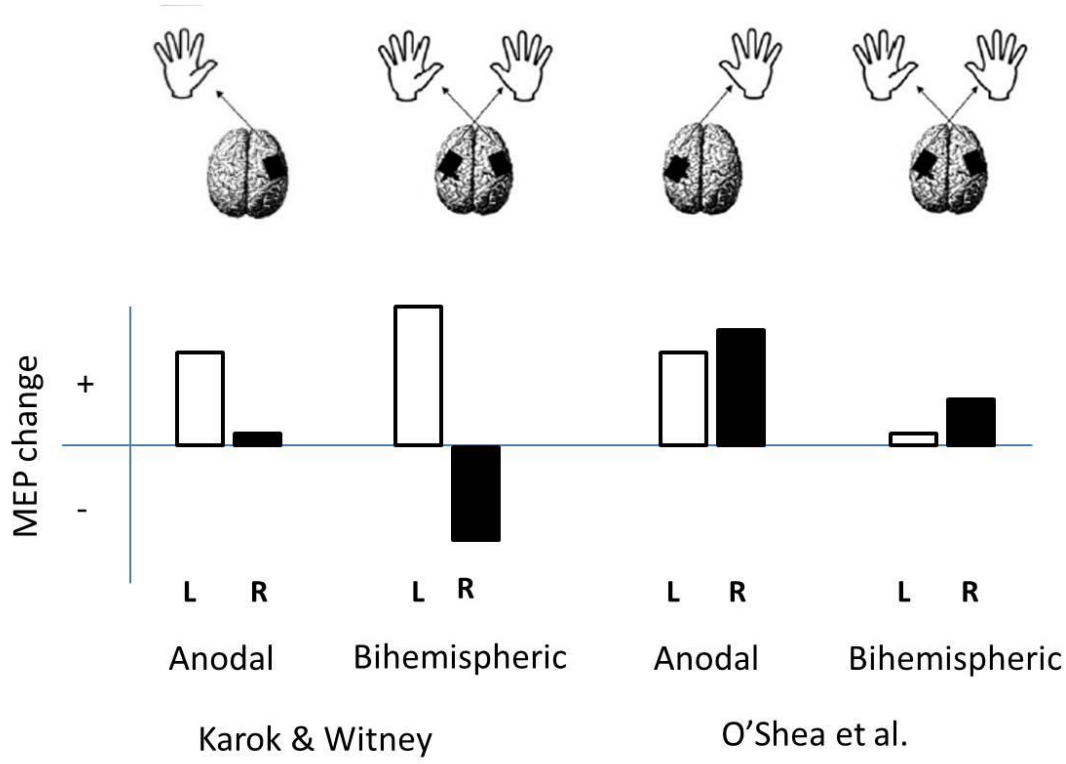


Figure 2.2 Representation of direction of MEP amplitude changes with anodal and bihemispheric tDCS. Adapted from Karok and Witney (2013) and O'Shea et al. (2014) Figure 1, page 928. L = left hand MEP, R = right hand MEP. Note, Karok and Witney applied the anode over right M1, O'Shea et al. applied the anode over left M1.

Differential effects of stimulating the M1 and PMd have been seen during implicit learning with the non-dominant (left) hand (Kantak et al., 2012). Anodal stimulation (1 mA, 8 cm^2 anode, 15 minutes) of either M1 or PMd improved learning over the trained blocks compared with sham (M1 significantly, PMd a tendency), but only M1 stimulation resulted in better retention of

learning 24 hours later. This suggests that modulation of M1 contributes to both online and offline learning with this task.

The effect of tDCS on motor learning over multiple days has been studied using the SVIPT (Reis et al., 2009) and a ballistic thumb movement task (Rroji et al., 2015). For the study by Reis et al., skill improved over five days of SVIPT training with the right hand for both anodal tDCS and sham, but the sham group showed negative offline learning (a decrement in skill between days) whereas the anodal group tended to show positive offline learning, which led to significantly better total learning for the active tDCS group. Performance remained higher three months later (Reis et al., 2009). Similarly, Rroji et al. (2015) found that although within-session learning of a ballistic thumb flexion task was independent of tDCS condition, performance one week later was improved with anodal tDCS compared to sham. This effect was not seen at the retention test the next day, and three blocks of training were performed for that retention test, so the authors speculated that the tDCS delivered during the initial training session somehow interacted with the practice during the testing the following day, resulting in better long term memory formation (Rroji et al., 2015). If confirmed, this could have implications for the use of tDCS in combination with rehabilitation as tDCS delivered on one day could impact on gains through physical therapy the next day. However, Prichard et al. (2014) found that although online training on a word/shape tracing task on two consecutive days was improved with anodal and bihemispheric tDCS compared to sham, performance gains with training, but without stimulation, on day three were not affected. Differences in findings may be the result of differing task characteristics and therefore this idea requires further investigation.

Older adults may show reduced or slower motor learning than young or middle aged adults (Boyd et al., 2008), and tDCS could help to overcome age related learning deficits (Zimerman et al., 2013). Using an explicit motor sequence learning task, Zimerman et al. (2013) found that learning was reduced for the older participants (55 – 88 years) in comparison with the younger

ones (22 – 31 years) during sham stimulation, but not during anodal tDCS due to improvements in performance for the older group which did not occur for the younger adults. There was a correlation between age and the amount of improvement with tDCS suggesting that the older the participant the more they benefited. This may be indicative of greater tDCS-induced improvements in motor network connectivity with increasing age, as ageing is associated with alterations in connectivity (Seidler et al., 2015; Vecchio et al., 2014) and improved connectivity correlates with improved motor performance (Seidler et al., 2015). Alternatively, there may be a ceiling effect for the younger subjects who were already competent at performing the task without neuromodulation. Younger adults may have shown improvements if they used their non-dominant hand which may be less dexterous. Further research is necessary to confirm these findings and determine whether cathodal or bihemispheric tDCS would have similar effects.

Changes in bimanual motor learning have been investigated using bihemispheric tDCS in a different manner (Gomes-Osman and Field-Fote, 2013). Stimulation was delivered with two anodes; one over each M1, prior to training of a repeating bimanual typing sequence over five consecutive days. Performance improved to a greater extent with active stimulation compared to sham. However, it was not clear whether improvements were made equally across hands and retention (one week later) was not affected by tDCS with this unique arrangement. The authors speculated that anodal-anodal tDCS increased M1 excitability of both hemispheres, but did not directly test this.

2.2.3 The influence of tDCS on motor learning after stroke

After stroke, motor skill learning is required both for functional recovery and for compensation (Krakauer, 2006) and this is the basis for rehabilitation strategies such as task specific training (Hubbard et al., 2009). Therefore, an understanding of the influence of tDCS on motor learning processes after stroke is vital if tDCS is to be considered as an adjuvant to rehabilitation.

In a series of experiments, the impact of stroke on implicit and explicit learning with the "unaffected" hand was investigated (Boyd and Winstein, 2001; Boyd and Winstein, 2003; Boyd et al., 2007). One study (Boyd and Winstein, 2001) demonstrated that stroke survivors lacked the ability to learn with the "unaffected" hand on the SRTT if they did not have explicit knowledge of the sequence. Their "explicit information" group were given details of the sequence to memorise, but they did not test whether simply being told of the existence of a repeating sequence was sufficient to promote learning. Their groups were small (n = 4 *per* group) and they did not report the functional level of any of the participants. In another study, the same authors argued that lesion location influenced motor learning ability, as patients with middle cerebral artery (MCA) stroke affecting the sensorimotor cortex were capable of learning implicitly but did not benefit from explicit information (Boyd and Winstein, 2003). Furthermore, in another study, Boyd et al. (2007) demonstrated that people with mild and moderate stroke impairments were capable of learning implicitly on the SRTT. This appears to contradict their earlier findings, which the authors did not attempt to explain. Together the studies suggest that improvements in motor preparation of the "unaffected" hand are possible in survivors of stroke, but that lesion characteristics and the information given to participants requires consideration.

Other authors have shown intact motor sequence learning with the SRTT (Exner et al., 2002) and a modified sequence learning task requiring patients to press buttons and turn dials with their "unaffected" hand (Pohl et al., 2001). With the button pressing task (Pohl et al., 2001), participants with mild stroke appeared to perform better than those with moderate stroke even though they were not using their paretic arm. This may suggest a global effect of stroke on the ability to learn a movement sequence. There were uneven sample sizes (9 moderate *vs* 18 mild) and the authors did not report whether the two groups were of similar ages which could affect learning ability (Boyd et al., 2008; Zimerman et al., 2013). Further, they did not include the first block of trials in their analysis, speculating that this block was just general learning effects, but it is possible that they missed a portion of the learning process. In a follow up study, Pohl et al.

(2006) confirmed that patients with mild or moderate stroke were capable of learning implicitly with their "unaffected" hand, but that those with moderate stroke had overall slower and more variable reaction times. Dovern et al. (2011) also used a modified SRTT where participants had to press large buttons rather than individual keys. They showed no difference between stroke survivors with or without apraxia and age-matched controls in the amount of implicit learning. However, they did not examine the rate of learning and once again all stroke survivors were using their "unaffected" hand. Therefore, it is not possible to draw any conclusions as to whether stroke affects the ability to learn movement sequences with the paretic upper limb which is typically the focus of physiotherapy. Further, they could only examine changes in response time, not changes in movement time or accuracy of movement with their task which could also be affected by learning. The use of accelerometers could potentially have negated this limitation.

The majority of studies examining the effect of tDCS on motor learning with the affected arm have tested stroke survivors who have just minimal upper limb impairment. Celnik et al. (2009) found that anodal tDCS combined with peripheral nerve (median and ulnar) stimulation improved retention of learning on the SRTT. Similarly, Zimerman et al. (2012) found that cathodal tDCS of the contralesional M1 facilitated online training compared with sham. Offline effects did not differ between conditions resulting in improved performance for the active condition at a retention test 90 minutes later. A subset of participants underwent TMS assessments, which revealed a decrease in contralesional M1 excitability for 60 minutes following stimulation, but no change in ipsilesional M1 excitability as would be hypothesised. This implies that the mechanism of improvements in movement following cathodal tDCS may not be exclusively due to an increase in ipsilesional M1 excitability from a reduction in trancallosal inhibition from the contralesional M1. However, SICI was reduced in the ipsilesional M1 and increased in the contralesional M1 immediately following stimulation and changes in SICI of the ipsilesional M1 correlated significantly with online task improvements, suggesting

that changes in inhibition may be the mechanism rather than excitability *per se*. Participants in both of these studies (Celnik et al., 2009; Zimerman et al., 2012) had mild impairment, which was necessary in order to perform the motor learning tasks which required individual digit key presses. Therefore it is important to be cautious about generalising these results to the wider stroke population, many of whom have more substantial upper limb impairment.

One study has examined the effect of tDCS on motor learning with moderately impaired stroke survivors (Lefebvre et al., 2012b). Their task was more suitable for this population, requiring participants to move a computer mouse to direct a cursor around a maze, whereby improvements in the speed-accuracy trade off were measured. Bihemispheric tDCS induced greater online improvements with the paretic arm than sham stimulation, and performance remained better when tested one week later. This study was a crossover within-subject design which helped to minimise potential confounds due to differences in learning ability that may be expected in a heterogeneous group of patients, and the task was more appropriate for people with impaired motor control due to stroke than the traditional motor learning tasks (SRTT and SVIPT). However, they did not attempt to compare their results with unilateral stimulation (anodal or cathodal). The authors reported that clinical characteristics (disability, age, stroke location) did not correlate with recall at one week, but did not report whether there were any correlations with online learning. Nevertheless, combined these studies indicate that tDCS has the potential to improve motor learning after stroke, and further research is needed with the paretic arm as the focus for people with a range of levels of upper limb motor impairment.

2.3 The effect of tDCS on upper limb motor function

2.3.1 Healthy Adults

There are numerous proof-of-principle studies examining improvements in motor performance and dexterity with tDCS in healthy adults (Hummel et al., 2010; Kidgell et al., 2013b; Matsuo et al., 2011; Parikh and Cole, 2014; Parikh and Cole, 2015). Hummel et al. (2010) revealed a significant moderate correlation between improvement in Jebsen Taylor Test (JTT) performance with active tDCS and age, indicating that older participants showed more pronounced effects, consistent with motor learning findings (Zimerman et al., 2013), see section 2.2.2. There was also a significant interaction between the type of JTT task (fine *vs* gross movements) and stimulation, as the fine motor subsections improved to a greater degree with active stimulation than the gross motor tasks. This could be the result of a ceiling effect for the less challenging gross motor tasks or it could be that tDCS somehow affects the control of the digits more than the gross movements that require larger and more proximal muscle groups.

Bihemispheric tDCS (anodal to non-dominant M1, cathodal to dominant M1) delivered during motor training of the non-dominant hand, with constraint of the dominant arm, has been shown to improve JTT performance in healthy young adults (Williams et al., 2010). TMS assessment revealed a decrease in corticospinal excitability for the dominant M1 with active stimulation and a decrease in IHI (dual coil paradigm) from dominant to non-dominant M1 as would be expected. However, there was no change in excitability of the non-dominant M1 or change in IHI from non-dominant to dominant. This may be unexpected given that the motor training was with the non-dominant hand and the anode placed over the non-dominant M1. However, these were healthy young adults and it is possible that decreases in excitability or inhibition are easier to detect with TMS than increases in this population. The neurophysiological changes correlated with functional improvements for the active tDCS group. The authors did not examine whether

the effects persisted beyond the training day, nor did they compare bihemispheric with unilateral tDCS.

Kidgell et al. (2013b) compared changes in Purdue pegboard test performance, MEP amplitude and SICI with anodal, bihemispheric or sham stimulation in young adults. They reported improved function of the non-dominant hand with both anodal and bihemispheric tDCS that persisted for at least 60 minutes as well as increased MEP amplitude and reduced SICI of right M1 for 30 minutes. Unlike the study by Williams et al. (2010) and a study with stroke survivors (Hummel et al., 2005), Kidgell et al. (2013b) found no correlation between changes in function and either MEP amplitude or SICI. However, they did not assess changes in TCI and the muscle that MEPs were recorded from (extensor carpi radialis longus) was in the forearm rather than a hand muscle which would be expected to be more crucially involved in the Purdue pegboard task. In a similar study, the same group of authors (Goodwill et al., 2013) compared electrode arrangements in healthy older adults (55 - 80 years, n = 11). Performance of a visuomotor tracking task requiring non dominant wrist extension movements improved with active tDCS immediately, but all conditions (including sham) were better than baseline when tested 30 minutes later. They provided participants with an initial familiarisation session in an attempt to eliminate motor learning, but it may be that they were still testing some elements of motor learning rather than motor performance only which may account for the improvements seen in the sham condition. Alternatively, these results may indicate that the rate of change may be more sensitive and susceptible to improvements with tDCS than total change in performance.

2.3.2 Stroke

As with healthy adults, there are many proof-of-principle studies investigating the effects of tDCS on upper limb function in people with stroke (Au-Yeung et al., 2014; Fusco et al., 2014b; Hummel et al., 2005; Hummel et al., 2006; Kim et al., 2009). A meta-analysis covering studies published up until 2012 indicated a small-moderate effect (Effect size (ES) 0.4 – 0.49) of anodal

tDCS on motor function (Butler et al., 2013), and a more recent meta-analysis (Chhatbar et al., 2016) found an overall moderate effect (ES 0.61) of tDCS on impairment. However, effectiveness may depend on the time since stroke, severity of impairment, electrode arrangement and the stimulation parameters (Chhatbar et al., 2016; Marquez et al., 2015).

In chronic stroke, improvements in JTT with anodal tDCS were shown to correlate with increases in corticospinal excitability (stimulus response curve slope) and decreases in SICI from ipsilesional M1 (Hummel et al., 2005). All participants had fairly low levels of impairment (Fugl-Meyer score > 91%) and the sample was small (n = 6) limiting the generalisability of their results. Nevertheless, this suggests that improvement in upper limb function with tDCS may be driven by changes in the balance between excitation and inhibition within M1.

Change in white matter integrity may also underlie improvements. Zheng and Schlaug (2015) used DTI to assess changes in the integrity of the corticospinal tract (CST) and "alternate motor tracts" in chronic stroke survivors following 10 sessions of bihemispheric tDCS and physical therapy. There was an increase in FA for the ipsilesional alternate motor tracts in the treated group indicating improved white matter integrity but no changes in the contralesional alternate motor tracts, either CST or in the untreated group (who received no tDCS or physical therapy). The FA changes in the ipsilesional alternate motor tracts correlated moderately with change in impairment. However, there was no sham tDCS group (i.e. physical therapy only), so it is unknown whether the increased FA is due to the tDCS, the physical therapy, or a combination of the two.

The interhemispheric imbalance model suggests that the stroke affected hemisphere is "doubly disabled", first by the loss of neurons due to the stroke itself, reducing activity of the ipsilesional M1, and secondly due to an increase in inhibition passed from the contralesional hemisphere which may be hyperactive (Murase et al., 2004; Takeuchi et al., 2010; Takeuchi and Izumi, 2012).

Evidence for the effectiveness of bihemispheric tDCS to reduce this hypothesised imbalance in excitability comes from studies with both acute and chronic stroke survivors (Bolognini et al., 2011; Di Lazzaro et al., 2014). Di Lazzaro et al. (2014) measured changes in MEP amplitude in acute stroke patients (≤ 4 days post-stroke) after one week of combined bihemispheric tDCS and constraint induced movement therapy (CIMT). Despite a lack of between-group differences in clinical outcomes, they found that the MEP laterality index shifted closer to zero (indicating no imbalance) at the three month follow up for the active tDCS group compared with sham. This appeared to be driven by both a decrease in contralesional M1 excitability and an increase in ipsilesional M1 excitability. Similarly, Bolognini et al. (2011) combined bihemispheric tDCS with CIMT in chronic stroke survivors (> 6 months) with moderate to severe hemiparesis and found improvements in function and impairment for the active group. This was accompanied by an increase in excitability for the ipsilesional M1, a decrease for the contralesional M1 and a reduction in IHI from the contralesional to the ipsilesional hemisphere. The neurophysiological changes correlated with the functional (JTT and Fugl-Meyer) changes.

However, there is also evidence that delivering tDCS based on the interhemispheric imbalance model may be ineffective. Increases in activity of the contralesional motor areas, and upregulation of ipsilateral pathways from the contralesional M1, may be compensatory, rather than maladaptive (Bradnam et al., 2013; Lotze et al., 2006; Ward et al., 2006; Ward et al., 2007). Therefore, attempting to reduce the excitability of the contralesional M1 may impair function in some patients (Ackerley et al., 2010; Bradnam et al., 2012), particularly where the ispilesional corticospinal tract has been severely disrupted by the stroke (Bradnam et al., 2012; Ward et al., 2006; Ward et al., 2007). Bradnam et al. (2012) examined the effect of cathodal tDCS of the contralesional M1 on selective proximal muscle activation in 12 stroke survivors (> 2 months post-stroke, mean 12 months). They found that although cathodal tDCS improved control for mildly impaired patients, it was worse for those with moderate to severe impairment. The authors speculated that ipsilateral projections from contralesional M1 may be facilitated during

recovery in people with impaired ipsilesional CST activity, resulting in a decrement in function when using cathodal tDCS to suppress the contralesional M1.

Anodal tDCS, of ipsilesional M1, may not be effective for severely affected patients either. Hesse et al. (2011) found that neither anodal nor cathodal tDCS improved function when combined with robot therapy in severely affected subacute patients (3 – 8 weeks post-stroke). They delivered tDCS for 20 minutes alongside robot therapy, five days *per* week for six weeks and measured FM at baseline, six weeks and three months. They did not test impairment throughout the six week training period so it is unknown whether the rate of improvement over the training could have been faster with active tDCS. Furthermore, they did not test bihemispheric stimulation. They did, however, suggest that those with subcortical stroke did improve to a greater degree with cathodal stimulation than those with cortical involvement. Combined, these studies highlight the importance of understanding who will respond to tDCS, and who will not, in order to tailor treatment to individuals.

Studies to systematically address the impact of electrode arrangement on the response to tDCS after stroke have drawn mixed conclusions. Studies using the JTT have shown a significant benefit of active stimulation over sham, but no significant differences between anodal (ipsilesional M1), cathodal (contralesional M1) and bihemispheric electrode arrangements (Fregni et al., 2005; Mahmoudi et al., 2011). However, these studies were limited by small sample sizes. Fregni et al. (2005) reported that although the difference between active electrode arrangements was not significant, the effect size for cathodal tDCS (ES 1.8) was greater than anodal (ES 1.2). Mahmoudi et al. (2011) reported that the magnitude of improvement was greater for bihemispheric tDCS than unilateral, whereas, Fusco et al. (2013) claimed that anodal tDCS was the most effective at improving performance on the nine hole peg test, and bihemispheric the least. Together these studies highlight the uncertainty as to the impact of

electrode arrangement and reinforce the need to have adequately powered studies to enable conclusions to be drawn.

The efficacy of bihemispheric tDCS was questioned in a study which aimed to predict the efficacy of bihemispheric tDCS based on the response to unilateral stimulation (O'Shea et al., 2014). Bihemispheric, anodal and cathodal tDCS were compared in a heterogeneous sample of chronic stroke survivors (range 1.5 – 5.8 years after stroke, aged 30 – 80 years, Fugl-Meyer score range 16 - 66) using a reaction time (RT) paradigm which required joystick wrist flexion movements when a green circle appeared on a computer screen. They also examined changes in GABA and Glx (a composite of glutamate and glutamine) in ipsilesional M1 using MRS. Performance deteriorated with sham stimulation, which was attributed to fatigue. This "fatigue effect" was improved by bihemispheric stimulation in participants with stroke affecting the left, but not right, hemisphere. However, for the group overall there was no change in task performance with bihemispheric tDCS, likely due to high inter-subject variability. Anodal and cathodal tDCS both improved RT significantly compared with sham. It is unclear why the hemisphere affected would affect the response to bihemispheric tDCS only. The response to anodal and cathodal stimulation (combined) was found to predict the response to bihemspheric tDCS, similar to findings within the same publication for MEP amplitude from healthy adults. The authors concluded that the bihemispheric montage produced weaker and more variable affects than unilateral, potentially due to differences in shunting of current across the scalp. They stressed the importance of gaining a better understanding of the differences between bihemispheric and unilateral tDCS in order to optimally design clinical trials. However, a recent meta-analysis (Chhatbar et al., 2016) found that bihemispheric tDCS had a larger effect on the change in impairment (ES 0.61) than either anodal (ES 0.21) or cathodal (ES 0.43). Therefore, the effect of electrode arrangement clearly needs further investigation with larger sample sizes including both left and right hemisphere strokes.

Although single-session studies provide useful proof-of-principle and mechanistic information, tDCS is likely to be used over multiple days or weeks in a clinical situation. Boggio et al. (2007) aimed to determine whether repeated sessions of tDCS would result in greater motor improvements than a single session. Weekly sessions of anodal (ipsilesional M1) or cathodal (contralesional M1) stimulation showed no additional benefit in JTT performance over that of a single session. However, the stimulation sessions did not include any motor practice component, and only four people took part. The majority of repeated session studies have delivered tDCS in combination with physical therapy more regularly; typically three to five times *per* week.

The effectiveness of repeated sessions of tDCS in acute or subacute stroke has been studied in small samples, with tDCS delivered over five to ten days (Di Lazzaro et al., 2014; Fusco et al., 2014a; Kim et al., 2010; Sattler et al., 2015), although the intensity and duration of stimulation varies between studies. Kim et al. (2010) delivered 10 days of anodal, cathodal or sham tDCS during occupational therapy in sub-acute stroke survivors (< 2 months post-stroke). They received tDCS (2 mA) for the first 20 minutes of each 30 minute session. Follow up Fugl-Meyer (FM) assessments were conducted one day after the intervention and at six months. The amount of occupational therapy delivered during the follow up period was recorded and did not differ between groups. Impairment (FM) was significantly better six months after cathodal tDCS in comparison with sham. The difference for anodal stimulation appears to be present, but did not reach significance and there were overall no differences between groups at the immediate post-test. Although the groups were small (6 anodal, 5 cathodal and 7 sham) and it is not possible to see how the rate of change in FM differed between groups throughout the six month period, the results suggested that cathodal tDCS may be effective at reducing impairment (Kim et al., 2010). Similarly, five days of anodal tDCS (13 minutes, 1.2 mA) combined with radial nerve electrical stimulation (0. 7 × motor threshold) was shown in acute patients (mean 5.5 days post-stroke) to improve JTT performance 30 days later (Sattler et al., 2015). However, there were no significant stimulation related differences for the nine hole peg test, grip strength, impairment (FM), or

neurophysiological measures (RMT or AMT). It is unclear why JTT performance would improve, but the nine hole peg test performance, which also involves dexterous movements, would not. There was no sham nerve stimulation condition included so it is not possible to know whether it added anything over and above the tDCS alone.

Khedr et al. (2013) compared anodal, cathodal and sham stimulation combined with standard physiotherapy in acute stroke patients (mean 17 days post-stroke), all of whom had good functional potential (Stinear et al., 2007). They delivered tDCS (25 minutes, 2 mA) prior to physiotherapy over six consecutive days and assessed global measures of disability; National Institute for Health Stroke Scale (NIHSS), Barthel index and the Orgogozo MCA Scale (OMCASS) as well as upper and lower limb strength measurements and motor thresholds using TMS. They found additional improvements with active stimulation over sham for OMCASS and Barthel index and a tendency for NIHSS, but no differences for hand grip strength or any differences between anodal and cathodal montages. The improvements were accompanied by a decrease in ipsilesional M1 RMT. Shoulder abduction, foot dorsiflexion and hip flexion strength also increased more for active tDCS than sham, indicating a potential lack of specificity to the tDCS effect. These results suggest that there may be no difference in efficacy between anodal and cathodal stimulation in the acute phase post-stroke. However, they did not use any specific upper limb functional ability assessments (e.g. FM, JTT or Action Research Arm Test) and the global measures of disability may not be sensitive enough to distinguish differences between the two stimulation types.

In contrast to the findings of Kim et al. (2010), Khedr et al. (2013) and Sattler et al. (2015), some studies show less promising results. Fusco et al. (2014a) delivered 10 days of cathodal tDCS (10 minutes, 1.5 mA) to the contralesional M1 of subacute stroke patients (< 30 days post-stroke) prior to inpatient rehabilitation. They completed an array of assessments, including the upper limb FM, nine hole peg test, timed up and go and the 10 m walk test. They found no effect of

stimulation on any of the parameters, although most showed improvement across time for both groups as would be expected. However, there were only 11 participants (5 active, 6 sham) and a large range in baseline upper limb impairment (FM range 4 - 66), with no assessment of potential for improvement (Stinear et al., 2012; Stinear et al., 2015). Despite the small sample size the authors have not presented data from individual participants which would help to aid interpretation. They also did not determine whether the hypothesised imbalance in cortical activity across hemispheres was present at baseline and chose to deliver the tDCS before, rather than during, rehabilitation. Therefore the conclusions should be taken cautiously. However, this null result is supported by Di Lazzaro et al. (2014) who demonstrated, in acute (≤ 4 days) patients, no between-group differences in clinical outcomes, including the NIHSS, the Action Research Arm Test (ARAT) or the nine hole peg test, following five days of bihemispheric tDCS either alone or in combination with CIMT. However Di Lazzaro et al. did find significant improvements in corticospinal excitability (increased ispilesional M1 excitability, decreased contralesional M1 excitability) for the active tDCS group. Once again the sample size was fairly small (n = 10 *per* group) and more than five days of treatment may be needed to induce measurable changes in motor function over physical training and spontaneous motor recovery alone.

Studies of chronic stroke survivors show equally mixed stimulation parameters and results, although meta-analyses indicate overall greater effectiveness than when delivered acutely (Chhatbar et al., 2016; Marquez et al., 2015). Bihemispheric tDCS, delivered for five days in combination with physical therapy (Lindenberg et al., 2010), or ten days with CIMT (Bolognini et al., 2011) was shown to improve impairment (FM), function (JTT and Wolf motor function test) and hand strength. Lindenberg et al. (2010) also demonstrated increased ipsilesional M1 and PMC activation for the active group during movement of the upper limb using FMRI and a significant correlation between changes in the laterality index of FMRI activation during movement and the change in Wolf motor function time for the active tDCS group only.

Unfortunately they did not test any longer term follow ups to see whether the effect persisted beyond one week.

Anodal and cathodal tDCS were compared when delivered in conjunction with modified CIMT over four weeks (Rocha et al., 2016). Stimulation was delivered at 1 mA for 13 (anodal) or 9 (cathodal) minutes, at rest. Modified CIMT consisted of immobilisation of the non-paretic upper limb for six hours *per* day (including weekends) and motor training with the paretic upper limb for one hour *per* day, three times *per* week. Assessments consisted of the FM, motor activity log and hand grip strength at baseline, immediately after the intervention and one month later. There were seven participants *per* group (anodal, cathodal, sham) and a significant increase in FM was found for the active tDCS groups, but not for sham. When comparing the change between active and sham stimulation, the improvement was significantly greater for anodal tDCS compared with sham, but cathodal failed to reach significance. All participants in the anodal tDCS group showed improvements in upper limb FM that exceeded the minimally clinically important difference, compared with five in the cathodal group and three in the sham group. This suggests that when combined with CIMT, in chronic patients, anodal tDCS may be more effective than cathodal, but larger sample sizes are required.

One of the appeals of tDCS is that it could potentially be used in the home setting. Five days of anodal tDCS, delivered in the home setting with occupational therapy, was shown to improve hand grip strength, but not JTT performance (Mortensen et al., 2016). The sample size was small (n = 7 sham, n = 8 anodal) and differences were not maintained one week later which may be because the duration of the intervention was too short. In contrast, Viana et al. (2014), who combined anodal tDCS with virtual reality (Wii) therapy three times *per* week for five weeks, found no additional improvements in impairment or function with anodal tDCS over sham. However, as with Fusco et al. (2014a), the tDCS was delivered before, rather than during, motor training which may have impacted results as tDCS has been shown to be ineffective at improving

motor learning if applied before task performance (Amadi et al., 2015; Stagg et al., 2011). Alternatively, it may be that tDCS provides additional benefit over motor training when the number of sessions is limited (e.g. 5 – 10 sessions) but that there are no additional enhancements over use-dependent plasticity alone when training occurs over five weeks or more.

2.4 Summary

The studies reviewed throughout this chapter suggest that tDCS of M1 can improve motor learning and upper limb function under certain conditions. However, there is variability in response within and between subjects and there is currently limited understanding as to who will respond to tDCS and who will not. There are inconsistencies across studies as to which tDCS electrode arrangement is utilised, with no clear determination of how the efficacy of tDCS is altered between unilateral and bihemispheric conditions. If tDCS electrode arrangement is found to differentially affect performance then this could have implications for the use of tDCS to improve physical function and rehabilitation. More research is therefore needed to understand how tDCS electrode arrangement impacts on changes in motor control for healthy adults and stroke survivors with upper limb impairment.

2.5 Aims of thesis

The overall aim was to further understand the role of electrode arrangement on the efficacy of tDCS in healthy adults and stroke survivors and to assess whether changes in motor sequence learning with tDCS relate to changes in transcallosal inhibition. Four experimental chapters are to follow.

Chapter 3: A motor sequence learning paradigm for stroke survivors with upper limb impairment.

The studies within this chapter aimed to evaluate a sequence learning paradigm that involved gross movements of the upper limb and to determine whether it had the potential to be used for the remaining studies.

Chapter 4: The effect of tDCS on motor sequence learning in healthy younger and older adults.

The aim of this study was to utilise the paradigm from Chapter 3 to assess the effect of tDCS electrode arrangement on motor sequence learning in healthy adults and to determine whether there is a differential effect based on age.

Chapter 5: The effect of electrode arrangement on motor sequence learning and upper limb function in chronic stroke.

This study aimed to assess the effect of tDCS electrode arrangement on both motor sequence learning and within-session change in upper limb function in stroke survivors.

Chapter 6: The effect of tDCS on retention of motor sequence learning.

The aim of this study was to determine the effect of tDCS electrode arrangement on the retention of motor sequence learning in healthy adults.

Chapter 3 A motor sequence learning paradigm for stroke survivors with upper limb impairment

3.1 Abstract

Background: Motor learning is required for learning new skills and for recovery from neurological injury, such as stroke. However, the ability of stroke survivors to learn a movement sequence is not well understood.

Aims: To develop and evaluate a motor sequence learning paradigm with the potential to be used by healthy adults and also stroke survivors with their paretic arm.

Methods: A sequence learning paradigm was developed involving gross movements of the arm to direct a computer mouse to illuminated targets on a monitor. Over three experiments healthy adults and stroke survivors were assessed to determine the pattern of learning on this task and differences between healthy adults and stroke survivors.

Results: Experiment one demonstrated the pattern of change in onset time (OT) over 25 repetitions of the movement sequence and found that learning was specific to the trained sequence. Experiment two demonstrated that learning improved after the first exposure to the paradigm, indicating that a familiarisation session is needed for repeated use. Experiment three found that stroke survivors were capable of learning the movement sequence, but showed impaired sequence specific learning compared with healthy age-matched controls.

Conclusions: This paradigm is suitable for use to assess motor sequence learning in healthy adults and stroke survivors with mild to moderate upper limb impairment.

3.2 Introduction

In life we are constantly learning and re-learning movements and skills and adapting to different situations. Motor learning involves the acquisition and refinement of new movement qualities, leading to long-lasting improvements in performance. An effective network of cortical and subcortical brain regions is required for motor learning to occur (Dayan and Cohen, 2011; Hikosaka et al., 2002; Karni et al., 1998; Lefebvre et al., 2015; Reis et al., 2008; Reis et al., 2009). After neurological injury, such as stroke, recovery of movement can be viewed as a form of motor learning where the damaged motor system is retrained to optimise the function of its remaining output. As such, motor learning forms the basis of functional rehabilitation strategies such as task specific training (Hubbard et al., 2009). Motor skills must be re-learned or movement patterns must be adapted to compensate for physical impairment. The ability of stroke survivors to re-learn movement patterns has clinical implications but is poorly understood.

One important component of successful motor control is the ability to learn sequential movement patterns. This is essential for many activities of daily living but some studies have demonstrated possible impairments in the ability to learn sequential movements after stroke (Boyd and Winstein, 2001; Boyd and Winstein, 2003; Boyd et al., 2007), whereas others have found that this ability is intact (Orrell et al., 2007; Pohl et al., 2001). However, most of the studies of people after stroke have focussed on motor learning with the "unaffected" rather than the paretic limb, even though true recovery requires improvements in the movement of the paretic limb (Krakauer, 2006). This may be, at least partly, because the traditional sequence learning tasks to experimentally assess motor learning (such as the SRTT) require dexterous movements of individual fingers to press keys. These movements are difficult or impossible for most stroke survivors with upper limb impairment, for whom the understanding of motor sequence learning capabilities is particularly important.

The ability to learn a movement sequence with the affected hand has been demonstrated (Zimerman et al., 2012) using an explicit key press sequence learning task. However, in that study all participants had mild impairment, scoring at least 61 (out of a possible 66) on the FM upper limb assessment (Fugl-Meyer et al., 1975). If motor sequence learning after stroke is to be better understood it is necessary to use a paradigm that enables performance with the paretic limb of patients with a range of functional impairments.

The purpose of this study was therefore to develop and evaluate a motor sequence learning paradigm that involved gross movements of the hand so that moderately impaired stroke survivors would be physically capable of performing the task with their paretic arm. The task encompasses elements from the SRTT (Nissen and Bullemer, 1987), other explicit and implicit key-press sequence learning paradigms (Stagg et al., 2011; Zimerman et al., 2012) and a modified SRTT (Moisello et al., 2009), by using a computer mouse to reach for illuminating targets on a computer monitor in a repeated order.

Specifically, this study aimed to test whether:

1. healthy adults would exhibit motor learning over 25 repetitions of the movement sequence,
2. motor sequence learning would be stable across multiple sessions if used in a repeated measures study design,
3. stroke survivors with upper limb impairment would be capable of performing the task with their paretic arm and demonstrate similar motor learning as age-matched healthy controls.

It was hypothesised that:

1. healthy adults would demonstrate significant sequence specific learning and that the pattern of learning would be similar to that observed with other sequence learning tasks,
2. motor sequence learning performance would remain constant across multiple sessions,
3. stroke survivors would demonstrate motor sequence learning but the rate would be slower than for healthy controls.

3.3 Methods

3.3.1 Participants

Participants were recruited through emails, advertisements, letters and word of mouth. All experiments were approved by the local Research Ethics Committee (BDM/11/12-35) and participants provided written informed consent. The healthy adults denied having any neurological conditions and used their non-dominant (left) hand to perform the task across all experiments.

3.3.1.1 Experiment One

Nine healthy adults, 4 male, mean age 32.9 years (range 28 - 42), completed one session of the motor sequence learning task. All were right handed, as assessed by the Edinburgh Handedness Inventory (mean laterality quotient 95 %; Oldfield, 1971).

3.3.1.2 Experiment Two

Nine healthy adults, 3 male, mean age 30.8 years (range 25 - 42), completed four sessions of the motor sequence learning task one week apart. All participants were right handed (mean laterality quotient 89 %; Oldfield, 1971). A different sequence was used each session, and the

order of sequences pseudo-randomised across participants. A subset of the participants (n = 7) had also participated in experiment one which formed the first session of this experiment.

3.3.1.3 Experiment Three

Twelve chronic stroke survivors, 10 male, mean age 64.2 years (range 39 - 80) and 10 age-matched healthy adults (6 male, mean age 66.0 years, range 50 - 85) attempted one session of the motor sequence learning task. Inclusion criteria for the stroke survivors were; aged > 18 years, first stroke at least 6 months prior with residual upper limb impairment. All stroke survivors used their affected hand to perform the task. Healthy adults were right handed (mean laterality quotient 87 %; Oldfield, 1971) and used their left hand to perform the task. Participants were given short breaks throughout the test if required. The characteristics of the stroke group are presented in Table 3.1, page 67.

3.3.2 Paradigm

The motor learning paradigm was a sequence learning task involving reaching movements of the hand, rather than key presses as for the traditional sequence learning tasks such as the SRTT. Participants sat at a table in front of a computer monitor (17 inch square) showing four grey circular targets (2.3 cm diameter) and a red central square (10.9 cm^2; Figure 3.1). The circular targets were all equidistant from the central square (8.5 cm). The number of targets chosen was consistent with other sequence learning tasks which involve four stimulus cues and button presses.

Participants held a computer mouse with their left hand (healthy adults) or their paretic hand (stroke survivors). This had been modified by removing the buttons, in order to facilitate performance with the paretic hand of stroke survivors who may have trouble controlling pressure on the mouse with the fingers. The programme was run using a customised Matlab (The Mathworks Inc., Massachusetts, USA) programme, which required input of an Excel file

(Microsoft 2007) that specified the sequence order. Participants initially moved the computer mouse to direct the cursor into the central square. One of the circular targets would illuminate (changing in colour from grey to white) 0.3 s after the cursor entered the central square, indicating that the participant should move the mouse to direct the cursor into the illuminated target. To ensure accuracy of movement, a dwell time in the target was imposed where the cursor had to remain there for 0.4 s before it would return to grey, indicating that they should return the cursor to the central square for illumination of the next target. This dwell time ensured that participants purposefully and accurately moved the cursor into the target, rather than sliding the cursor through it and out the other side.

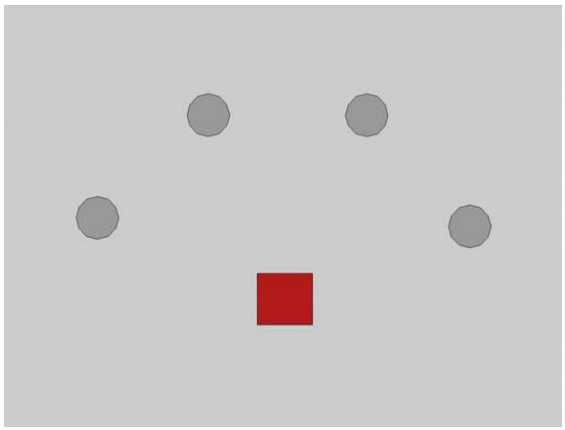


Figure 3.1 Representation of motor sequence learning programme as shown on the computer monitor. One central square and four circular targets can be seen.

Values for onset time (OT), movement time (MT) and path length (PL) were automatically computed by the programme and saved into the same Excel file as where the sequence was specified, to enable offline analysis. OT was calculated as the time, in seconds, from when the target illuminated to when the cursor left the central square. Since there was a delay of 0.3 s between the cursor entering the central square and illumination of the target, it was possible to calculate a negative OT if participants anticipated the next target and moved the cursor towards it prior to illumination. The target would illuminate even if the cursor had moved out of the central square but would not illuminate if the cursor had not entered the central square after the previous target turned off. This ensured that participants moved the cursor through the

central square each time and would have the same distance to move the cursor to reach each target even if they anticipated it. MT was automatically calculated as the time, in seconds, from the cursor leaving the central square to arriving in the illuminated target (providing the cursor remained in the target for at least the length of the dwell time). PL was calculated as the number of pixels the cursor travelled through to get from the central square to the illuminated target, and as such an increased PL represents reduced accuracy of movement.

3.3.3 Setup

Participants were seated ~60 cm in front of the computer monitor, with the computer mouse on the table in front of them. A sequence of 10 movements was used, chosen to be consistent with previous sequence learning studies which have 10 key presses (Nissen and Bullemer, 1987; Stagg et al., 2011). The non-dominant hand was chosen for healthy adults as the use of a computer mouse with the non-dominant hand was novel and considered difficult, whereas the majority of healthy participants were familiar with the use of a computer mouse with their dominant hand. The stroke survivors used their paretic hand to move the mouse which was novel and difficult.

Two practice sequences were completed initially (a third was possible for stroke survivors if required) to familiarise the participants with the use of the computer mouse and the movement of the cursor to the targets. This paradigm was used as an explicit learning task. Therefore, participants were informed that they would repeat the same sequence of 10 movements, 25 times, and that they could anticipate target appearance if they knew which would illuminate next. The sequence for each participant and session was chosen randomly from a pool of eight sequences. Targets were not numbered but if numbers were assigned left to right then examples of the sequences include; 1-2-2-4-3-1-3-4-1-2, 3-1-2-4-1-4-3-1-2-4 and 2-3-3-1-4-2-3-1-3-4. Pilot experiments showed no noticeable differences in difficulty between the sequences (data not shown). Following completion of the 25 repetitions of the sequence, a random sequence (10

movements) was performed to distinguish between changes due to general learning and sequence specific learning effects.

3.3.4 Analysis

3.3.4.1 General

The OT, MT and PL values were automatically generated for each movement of the sequence. Speed of cursor movement (pixels.s^{-1}) was calculated manually using Excel, by dividing PL by MT for each movement. The median value of each variable was calculated for each of the 25 repetitions. The median of the 10 movements was used, rather than the mean, as it is less likely to be skewed by outliers when there are only 10 values recorded for each repetition.

To enable quantification of learning in a manner that would take into account both the rate and the amount of change, the area under the curve (AUC) was calculated using values normalised to the first repetition of the sequence, using the following formula:

$$(1) \qquad AUC = \sum \tfrac{1}{2} (r_{i+1} - r_i) (v_i + v_{i+1})$$

where r = repetition number and v = value.

If values did not change over 25 repetitions then a value of 24 would result for the AUC. If values reduced then AUC would be < 24. Therefore, motor sequence learning is evident as an AUC < 24 for OT, with smaller values indicating improved rate and/or amount of learning. An improvement in movement accuracy would be evident by a PL AUC < 24, and an increase in movement speed by an AUC > 24. This measurement provides additional information about the rate of learning compared with simply examining the change between the first and the last repetition of the repeated sequence. For example, two individuals could show the same change in OT between the first and last repetition, but the first could reach a plateau earlier through the

25 repetitions than the second. Individual one would then show a smaller AUC, indicating improved learning over individual two.

To reduce the number of statistical comparisons OT, PL and speed values were averaged across consecutive repetitions (i.e. 2-3, 4-5 etc.) forming 13 blocks of values rather than 25 repetitions. Herein the term "block" refers to these average values.

Statistical analysis was performed using SPSS 21.0 (IBM Inc.). Normality of the standardised residuals was assessed using Shapiro Wilk tests and visual inspection of frequency histograms. If the assumption of normality was sustained then parametric statistics were utilised, otherwise if transformation was ineffective then non-parametric statistics were used. Violations of the sphericity assumption were corrected using the Greenhouse-Geisser correction if Epsilon < 0.75 and the Huyn-Feldt correction if Epsilon > 0.75. Significance was $p < 0.05$. *Post-hoc* pairwise comparisons were conducted with a modified Bonferroni correction for multiple comparisons (Rom, 1990). Data are presented as individual subjects to aid interpretation or as mean ± standard error of the mean (SEM) unless otherwise specified.

3.3.4.2 Experiment one specific analyses

One way repeated measures analysis of variance (rmANOVA) was used to test for the effect of BLOCK on OT, PL and speed. To test for sequence specific learning effects, planned comparisons using paired samples t-tests were conducted comparing values from the random sequence block with those of the last block of the repeated sequence.

3.3.4.3 Experiment two specific analyses

One way ANOVAs were used to test for the effect of SESSION on the OT (log transformed) and PL of the first repetition and for the effect of SESSION on the AUC for OT, PL and speed. Friedman tests were used to test for the effect of SESSION on the speed of the first repetition and the OT

difference between the last block of the repeated sequence and the subsequent random sequence. Additionally, an “anticipation” was recorded if the cursor left the central square prior to target illumination. The total number of accurate anticipations was recorded for each participant and a Friedman test used to assess whether there was an effect of SESSION.

3.3.4.4 Experiment three specific analyses

One way rmANOVAs or Friedman tests were used to test for the effect of BLOCK on OT, PL and speed for stroke and control groups separately. To test for sequence specific learning effects, planned comparisons using paired samples t-tests or Wilcoxon Signed Rank tests were conducted comparing values from the random sequence with those of the last block of the repeated sequence.

To compare groups, independent Sample t-tests or Mann Whitney U tests were used for the initial values for OT, PL and speed to determine whether baseline task performance differed between stroke survivors and healthy age-matched control participants. To test for differences in motor sequence learning a 12 BLOCK × 2 GROUP mixed rmANOVA was conducted using OT values that were normalised to the first repetition of the sequence. Additionally, independent samples t-tests or Mann Whitney U tests were used to compare values for the AUC for OT, PL and speed and for the normalised OT difference between the random sequence and the last block of the repeating sequence (sequence specific learning).

3.4 Results

3.4.1 Experiment One

The task took participants on average 17 minutes to complete (range 13-21 minutes). All participants completed the session.

3.4.1.1 OT

The rmANOVA revealed a significant effect of BLOCK ($F_{3.1,24.6} = 23.81$, $p < 0.001$) indicating that OT decreased with repetition of the sequence (Figure 3.2A). Pairwise comparisons showed that OT was significantly lower than the first repetition for all subsequent blocks ($p \leq 0.005$) indicating rapid learning. Planned comparisons revealed that the OT for the last block (mean = 0.008 ± 0.06 s) was significantly lower than the subsequent random block (mean = 0.38 ± 0.03 s; $t(8) = -5.905$, $p < 0.001$).

3.4.1.2 PL

The rmANOVA revealed no effect of BLOCK ($F_{4.4,35.1} = 1.629$, $p = 0.185$) indicating that movement accuracy did not change with practice (Figure 3.2B). Similarly, planned comparisons indicated no difference between the random block and last block of the repeated sequence ($t(8) = -0.972$, $p = 0.360$).

3.4.1.3 Speed

The rmANOVA revealed an effect of BLOCK ($F_{10.9,86.9} = 3.015$, $p = 0.004$) as speed increased with repetition of the movement sequence (Figure 3.2C). Pairwise comparisons showed that speed was significantly greater than the first repetition for blocks 9 ($p = 0.046$) and 13 ($p < 0.001$). There was a tendency also for blocks 7 ($p = 0.037$), 10 ($p = 0.015$) and 12 ($p = 0.016$), but these were not significant with the modified Bonferroni correction. Planned comparisons indicated that the speed of the last block (1090 ± 40 pixels.s^{-1}) was significantly faster than the subsequent

random block (980 ± 51 pixels.s^{-1}; p = 0.030), suggesting sequence specific improvements in motor control.

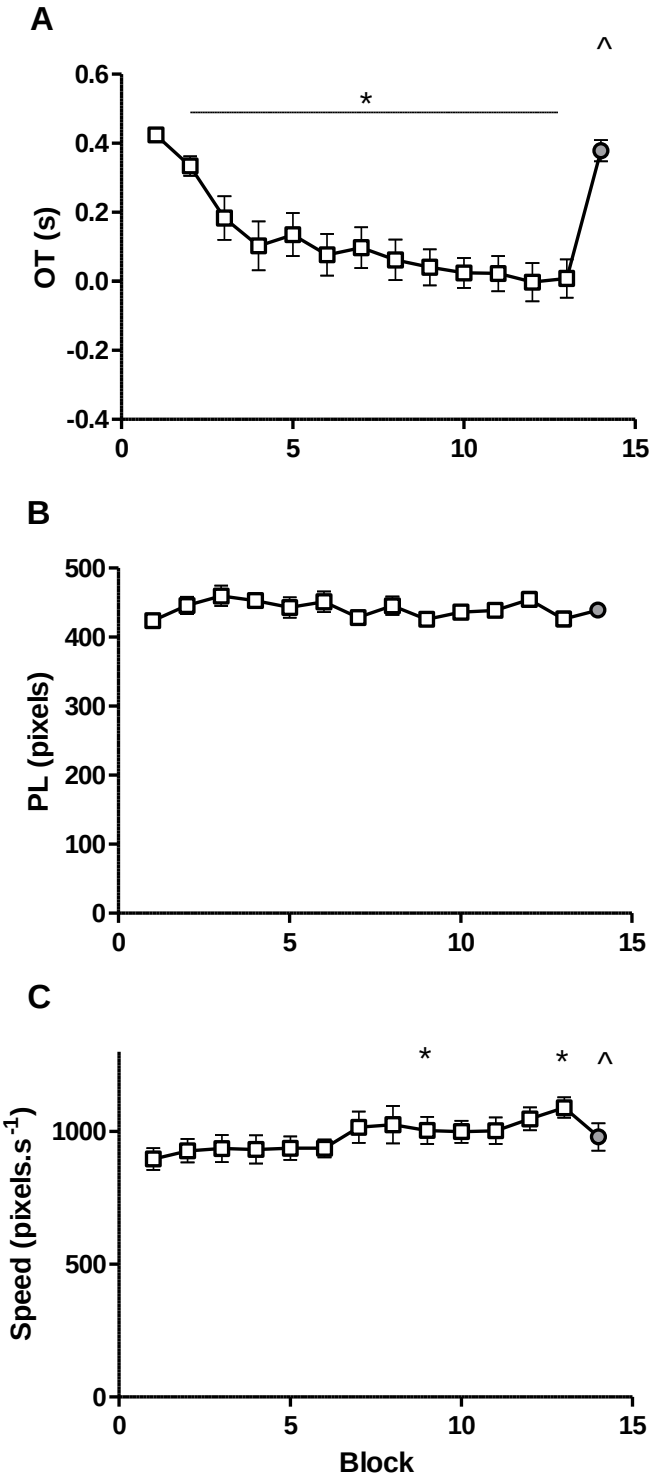


Figure 3.2 A OT, **B** PL and **C** Speed (mean ± SEM) in 9 healthy adults.
Open squares are for repeated sequence and filled (grey) circle (block 14) is for the random sequence.
* significant difference from first repetition p < 0.05, ^ significant difference to last block of repeated sequence, p < 0.05.

3.4.2 Experiment Two

3.4.2.1 OT

Initial OT (i.e. reaction to target illumination) was not normally distributed and so a log transformation was applied. The rmANOVA revealed a tendency toward an effect of SESSION ($F_{3,24}$ = 2.884, p = 0.057; non-transformed mean: Session (S) 1 = 0.41 ± 0.01 s, S2 = 0.39 ± 0.04 s, S3 = 0.38 ± 0.03 s, S4 = 0.35 ± 0.04 s).

The rmANOVA revealed an effect of SESSION on the OT AUC ($F_{3,24}$ = 3.027, p = 0.049) which was less for the third session than the first (p = 0.035). Figure 3.4A shows the reduction in OT over the 13 blocks for each session and Figure 3.3B shows the AUC values for each participant in each session. Lower values indicate better reduction in OT across the repetitions (improved learning).

The Friedman test revealed an effect of SESSION on the OT difference between the last block of the repeated sequence and the random block (p = 0.031). The OT difference was significantly lower for the first session compared with the forth (p = 0.038, Figure 3.3C), indicating reduced sequence specific learning in the first session. There was a similar tendency between the second and forth sessions (p = 0.051).

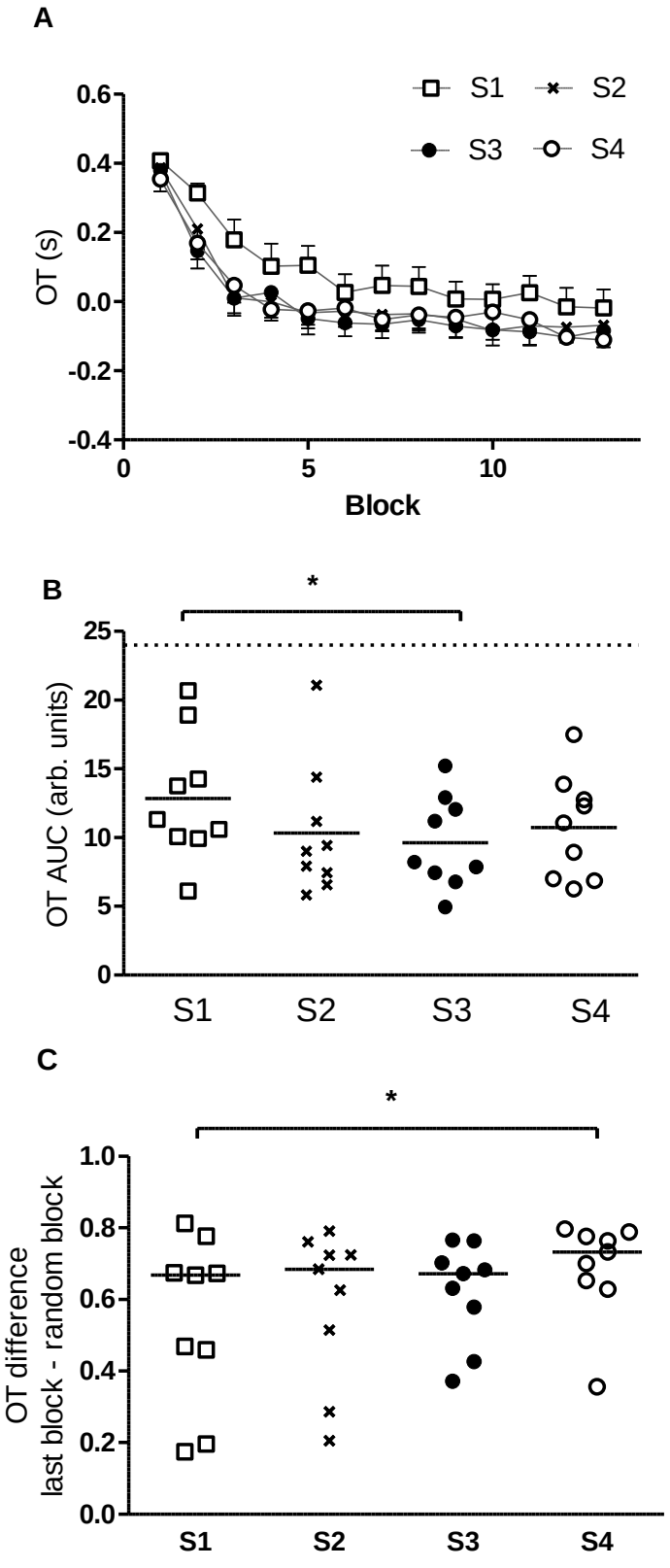


Figure 3.3 A. OT over the blocks for each of the four sessions (mean ± SEM).
B. OT AUC for individual participants. Solid horizontal lines represent mean, dotted horizontal line indicates AUC if no change in OT occurs. **C.** OT difference between last and random blocks for individual participants. * There was an effect of session, $p < 0.05$. S = session.

3.4.2.2 PL

There was no effect of SESSION on the initial PL ($F_{3,24}$ = 0.955, p = 0.43), indicating that the baseline task accuracy did not change (S1 = 424 ± 10 pixels, S2 = 445 ± 11 pixels, S3 = 430 ± 13 pixels, S4 = 431 ± 12 pixels). Similarly, there was no effect of SESSION on the PL AUC ($F_{3,24}$ = 2.657, p = 0.071).

3.4.2.3 Speed

The Friedman test showed no effect of SESSION on the initial speed (p = 0.71), indicating that the baseline task performance did not change (Median: S1 = 918 pixels.s^{-1}, S2 = 1040 pixels.s^{-1}, S3 = 981 ± 73 pixels.s^{-1}, S4 = 1001 pixels.s^{-1}). Similarly, the rmANOVA showed no effect of SESSION on the speed AUC ($F_{3,24}$ = 0.293, p = 0.83).

3.4.2.4 Number of anticipations

The Friedman test showed an effect of SESSION on the number of accurate anticipations (p = 0.019, Figure 3.4). Pairwise comparisons showed that session one had significantly less anticipations than all other sessions (p < 0.02) but there were no significant differences between sessions 2, 3 and 4 (p > 0.5).

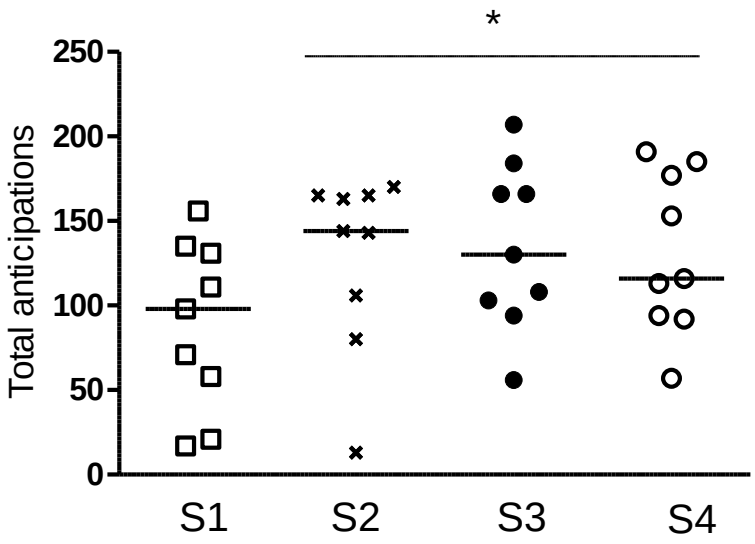


Figure 3.4 Total number of anticipations for each session for individual participants.
Solid horizontal lines represent median, S = session. * significant difference from session 1, p < 0.05.

3.4.3 Experiment Three

The task took on average 22 minutes for stroke survivors (range 15 – 36 minutes) and 15 minutes for controls (range 13 – 20 minutes). Two of the stroke participants (#9 and 10; Table 3.1) were unable to complete all 25 repetitions of the sequence. Participant #9 found it too difficult to keep the mouse on the table while moving it and therefore the cursor did not move properly on the screen, and participant #10 found it too tiring to concentrate on the screen (due to poor eyesight). Their data was not used for analysis.

3.4.3.1 Stroke Survivors

OT

The rmANOVA revealed a significant effect of BLOCK ($F_{3.2,29.0} = 4.560$, $p = 0.009$) and pairwise comparisons indicated that OT was lower than the first repetition for block 3 ($p = 0.045$). There was a tendency for a difference for blocks 4, and 7 – 13 ($p < 0.05$) which did not reach significance with the modified Bonferroni correction (Figure 3.5A, page 70).

Planned comparisons indicated that the OT for the last block (mean = 0.33 ± 0.07 s) was significantly lower than the subsequent random sequence (mean = 0.50 ± 0.04 s; $t(9) = -2.882$, $p = 0.018$) indicating specificity of OT improvements to the trained movement sequence.

PL

The Friedman test showed no effect of BLOCK ($p = 0.577$) indicating that movement accuracy did not change with practice. Similarly, there was no difference between the random sequence and the last block of the repeated sequence ($p = 0.646$).

Table 3.1 Characteristics of stroke survivors participating in experiment three.

Patient	Sex	Affected Arm	Time since stroke (mo)	Age (years)	FM (max 66)	Lesion Type	Lesion Location
1	M	L	57	60	40	I	Internal Capsule
2	M	R	20	49	48	H	Parietal, Precentral Gyrus
3	F	L	18	67	40	I	Lacunar
4	F	L	47	80	60	I	Cerebellar
5	M	R	138	65	59	H	Internal Capsule
6	M	R	16	61	39	I	Pons
7	M	R	76	76	58	I	MCA territory
8	M	R	118	66	39	I	Pons
9[a]	M	L	27	57	35	I	Thalamus
10[a]	M	R	11	74	57	H	Basal Ganglia
11	M	L	10	76	59	I	Parietal
12	M	L	54	39	40	H	Putamen, Right lateral Ventricle

M = Male, F = Female, L = left, R = right. mo = months. FM = Fugl-Meyer upper limb assessment score. I = Ischaemic, H = Haemorrhagic. MCA = middle cerebral artery.

[a] did not complete all 25 repetitions of task and data were not used for analysis.

Speed

The rmANOVA showed no effect of BLOCK ($F_{4.0,35.8}$ = 0.924, p = 0.461) indicating no change in speed with practice. Similarly the random sequence was not significantly different to the last block of the repeated sequence (p = 0.646).

3.4.3.2 Age-matched controls

OT

The rmANOVA revealed an effect of BLOCK ($F_{3.2,28.6}$ = 12.141, p < 0.001) and pairwise comparisons indicated that OT was significantly lower than the first repetition for blocks 3 and 6 – 13 (p < 0.05). There was a tendency for blocks 2, 4 and 5 also (p < 0.05), which did not reach significance with the modified Bonferroni correction (Figure 3.5A, page 70).

Planned comparisons indicated that the OT for the last block (mean = 0.078 ± 0.06 s) was significantly lower than the subsequent random sequence (mean = 0.421 ± 0.03 s; t(9) = -6.601, p < 0.001) indicating that OT improvements were specific to the trained movement sequence.

PL

The Friedman test showed no effect of BLOCK (p = 0.466) indicating that accuracy of movement did not change with practice. Similarly, planned comparisons indicated no difference between the random sequence and the last block of the repeated sequence (p = 0.721).

Speed

There was no effect of BLOCK ($F_{4.0,36.3}$ = 1.097, p = 0.373) indicating that movement speed did not change with practice. Similarly, planned comparisons indicated no difference between the random sequence and the last block of the repeated sequence (t(9) = -1.078, p = 0.309).

3.4.3.3 Comparison between stroke survivors and age-matched controls

There were significant differences between the stroke and control groups for baseline (first repetition) OT ($t(18) = 5.455$, $p < 0.001$) and speed ($t(18) = -3.819$, $p = 0.001$), with stroke survivors showing longer OT, and slower movement, but no differences in accuracy (PL; $p = 0.17$). Initial values are shown in Table 3.2

Table 3.2 Initial values for stroke survivors and control groups.

	Stroke mean (SD)	**Control** mean (SD)
OT (s)	0.56 (0.06)*	0.42 (0.06)
PL (pixels)	570 (229)	442 (69)
Speed (pixels.s^{-1})	557 (168)*	877 (205)

OT = onset time, PL = path length. * significant difference between groups, $p < 0.05$.

Since there was a difference between groups for baseline (first repetition) OT, the OT of each block was normalised to the first repetition of the sequence for additional analyses (Figure 3.5B). The rmANOVA revealed a significant effect of BLOCK on normalised OT ($F_{4.2,76.4} = 12.604$, $p < 0.001$), but no effect of GROUP ($F_{1,18} = 2.357$, $p = 0.142$) or BLOCK by GROUP interaction ($F_{4.2,76.4} = 1.321$, $p = 0.269$), indicating that there was no detectable difference in the pattern of learning across groups (Figure 3.5B). Similarly, the AUC did not differ between groups for OT ($t(18) = 1.52$, $p = 0.146$;Figure 3.5C), PL (Mann Whitney U Test; $p = 0.48$) or speed ($t(18) = 1.159$, $p = 0.2$;Figure 3.5C).

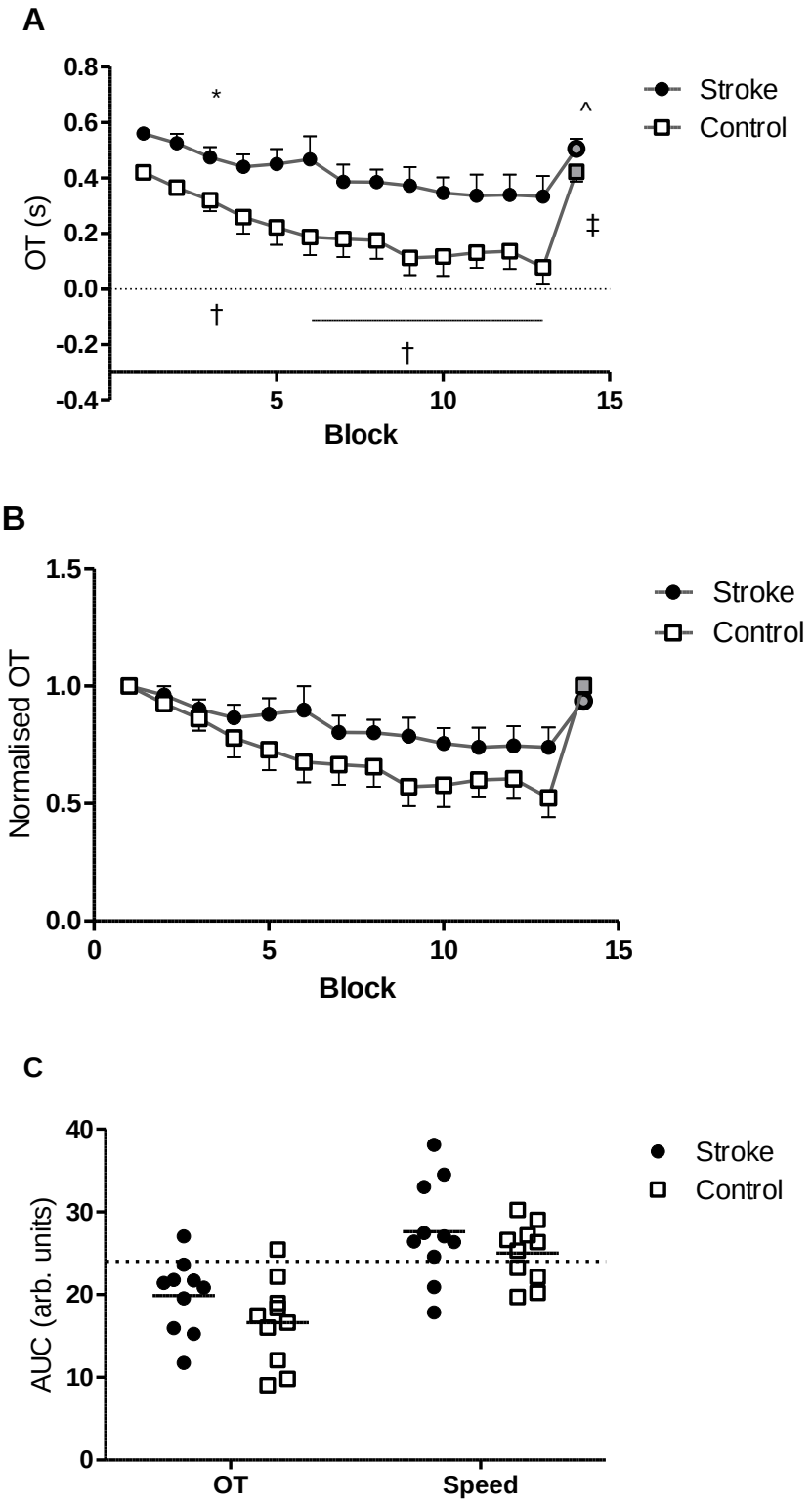


Figure 3.5 A OT for stroke survivors (mean ± SEM; filled circles) and controls (open squares). Block 14 (grey symbol) represents the random sequence. * and † significant difference from first repetition for stroke and control groups respectively, $p < 0.05$; ^ and ‡ significant difference to last block for stroke and control groups respectively, $p < 0.05$. **B** OT normalised to first repetition for stroke survivors (mean ± SEM; filled circles) and controls (open squares). **C.** Area under the curve (AUC) for individual participants for OT and Speed. Filled horizontal lines indicate mean, dotted horizontal line indicates AUC if no change occurred. There were no significant differences between groups.

The difference in normalised OT between the last block of the repeated sequence and the random sequence was larger for the control group than the stroke participants (p = 0.015) indicating that the sequence specific learning differed (Figure 3.6).

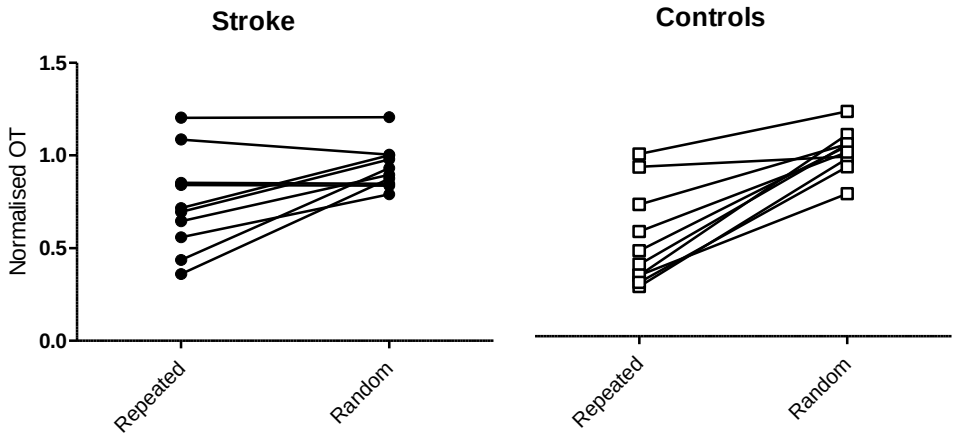


Figure 3.6 Normalised OT for last block of repeated and random sequence for individual participants. There was a significant difference between groups for the difference in normalised OT (p = 0.015).

3.5 Discussion

These experiments demonstrate the pattern of motor sequence learning with this gross movement paradigm in healthy adults and chronic stroke survivors with moderate to mild upper limb impairment. All groups were capable of learning the movement sequence, evidenced by a significant reduction in OT across blocks. The pattern of reduction in OT appears similar to that of reaction time shown with other sequence learning paradigms (Moisello et al., 2009; Nissen and Bullemer, 1987; Stagg et al., 2011).

3.5.1 Healthy young adults exhibit motor sequence learning

Experiment one revealed that OT reduced with practice for healthy young adults, in a sequence specific manner, as subsequent performance of a random sequence resulted in a significantly greater OT than the last block of the repeated sequence (Figure 3.2A, page 62). This is consistent with the traditional SRTT paradigm (Nissen and Bullemer, 1987) which assesses implicit learning. There was a significant increase in speed, but no change in PL indicating that the increase in movement speed across blocks was not at the expense of reduced accuracy. Like the changes in OT, the improvements in movement speed were specific to the trained sequence, as the speed for the subsequent random sequence was significantly slower than the last block of the repeated sequence. This indicates that the ability to improve both preparation and performance of movements was associated with the learning of the sequence, rather than familiarisation with the task apparatus.

Lefebvre et al. (2012a) demonstrated improvements in the speed-accuracy trade-off for healthy adults while performing a “circuit learning” paradigm. They found that learning was accompanied by activation of a network of brain regions including M1, PMC, SMA, thalamus, putamen, cerebellum and oculomotor and visual areas. Although their paradigm was not a sequence learning task *per se* the movement of the mouse was similar to the current task and

therefore it is likely that improvements in speed with learning on the current task would involve a similar network of neural activity.

3.5.2 Change in performance occurs after the first session

Experiment two aimed to determine whether the rate of learning would be stable across multiple sessions allowing the task to be utilised in a future repeated measures study design, e.g. to assess the effect of non-invasive brain stimulation on motor sequence learning. The purpose was not to examine retention of the learned sequence and therefore a different movement sequence was used in each session. There was a tendency toward an effect of session on baseline (non-normalised) OT, indicating that ability to react to the illuminated target tended to improve after the first session. In contrast, no effect of session was seen for baseline PL or speed suggesting that these aspects of task performance remained constant across the four sessions. There was an effect of session on OT AUC, (Figure 3.3B, page 64), indicating that total learning improved after the first session. Sequence specific learning also improved, as the difference in OT between the last block of the repeated sequence and the subsequent random block was significantly larger for the fourth session in comparison with the first (Figure 3.3C, page 64). These OT differences were accompanied by significantly less anticipations in the first session than the remaining sessions, but no differences between sessions two, three and four (Figure 3.4, page 65). Together, these findings indicate that motor sequence learning improves after the first exposure to the task, but remains stable for subsequent sessions performed one week apart. This is likely due to familiarisation with the task and the participants developing a strategy to learn the movement sequence. This is consistent with the findings of Pohl et al. (2006) who showed initial improvements in reaction time with random sequences and a reduction in variability, likely due to familiarisation of the task components. Therefore, if used in a repeated measures study, participants should be provided with a familiarisation session to ensure that any initial improvements are discounted and do not influence results.

3.5.3 Similarities and differences between stroke survivors and healthy controls

Experiment three aimed to determine whether this sequence learning paradigm would be suitable for use with the paretic arm of stroke survivors with moderate and mild upper limb impairment and would therefore be relevant for rehabilitation studies. The FM scores ranged from 35 to 60, indicating that all participants were more impaired, and therefore more representative of the target stroke population, than those studied previously by Zimerman et al. (2012) with their key press sequence learning task. Although the sample size was small, significant improvements in OT were seen, without a reduction in speed or a decrement in accuracy. There were no differences in the AUC between groups for OT, PL or speed. This is consistent with the previous studies that have shown intact motor learning ability in stroke survivors (Orrell et al., 2007; Pohl et al., 2001) and extends their findings to include overall learning with the paretic arm. As with healthy adults (both young and older) the improvements in OT for the stroke group as a whole showed specificity to the trained sequence. However, there was a significant difference between stroke survivors and controls for the OT difference between the last block of the repeated sequence and the subsequent random sequence indicating that impairment in sequence specific learning was evident for the stroke group. All healthy adults demonstrated a slower OT for the random sequence than the trained sequence, whereas some stroke survivors did not (Figure 3.6, page 71).

The reason underlying the impairment in sequence specific learning after stroke is not known. This is the first study comparing healthy controls with stroke survivors using their paretic arm while performing a motor sequence learning task. It could therefore be due to functional limitations of the stroke survivors, if they were not physically able to move the cursor out of the central square any quicker. Alternatively, it may be that stroke survivors have reduced plasticity and improvements in movement preparation occur more slowly than healthy controls. It could also indicate a cognitive or motor sequence learning impairment specifically due to the stroke

which limits the maximum improvement achievable. These possibilities warrant further investigation.

The sample was heterogeneous in terms of lesion location so it is currently unknown whether this might underlie why some participants showed a slower OT for the random sequence whereas others did not. One of the participants who showed no difference between the last block of the repeated sequence and the random sequence had an ischaemic stroke that affected the cerebellum. Boyd and Winstein (2004a) demonstrated that improvements in spatial accuracy with implicit learning were preserved in people with cerebellar stroke but improvements in temporal accuracy were not. Since OT is a temporal parameter, this participant's result might therefore extend their findings to suggest that temporal changes with explicit learning could also be affected by cerebellar stroke. Further, the cerebellum is known to play a role in feedforward control, which would be necessary in order to prepare movements to anticipate target appearance with learning. Two other participants showing impaired learning had haemorrhagic strokes affecting subcortical structures (basal ganglia and internal capsule). The putamen and thalamus have been found to be involved in both sensorimotor and sequence learning paradigms (Hardwick et al., 2013) as well as the "circuit learning" paradigm (Lefebvre et al., 2012a), and the provision of explicit information has been found to impair implicit learning in people with basal ganglia lesions (Boyd and Winstein, 2004b). Although these are all individual participants, their learning deficits may be therefore directly related to the location of their strokes. If confirmed then this would have potential implications for rehabilitation as motor learning principles underlie physiotherapy techniques such as task specific training (Hubbard et al., 2009). Stroke survivors may need more repetitions of a task to ensure adequate improvements. Reduced specificity of learning may also potentially be beneficial if it means that training on one task might overlap to another untrained movement.

3.5.4 No change in speed or accuracy of movement

Neither the speed of movement nor the accuracy changed for either the stroke group or the healthy older adults, although increases in movement speed were evident for healthy young adults in experiment one (Figure 3.2C, page 62). This would suggest that for older adults the improvements with learning were solely due to preparatory effects on OT. Older adults may need to focus primarily on the time to leave the central square whereas the younger adults may have been able to split their attention to learning the task and increasing their movement speed. Alternatively it may indicate that improvements in movement speed do not accompany sequence learning in older people. However, the older and younger groups have not specifically been compared in this chapter so these possibilities are just speculation which will be addressed in Chapter 4.

Accuracy (PL) was not found to change in any of the experiments. This may indicate that the path length measure is not sufficiently sensitive to detect improvements in accuracy, which would be expected to be quite small. Stroke survivors had slower baseline movement speed than healthy controls and therefore may have sacrificed speed in order to focus on learning the movement sequence and maintaining accuracy.

The slower initial OT and speed evident for stroke survivors may be a general effect of stroke, rather than specifically related to the use of the paretic hand, as previous studies of motor learning with the hand ipsilateral to the lesion have also indicated slower performance in stroke participants compared with controls (Orrell et al., 2007; Pohl et al., 2001; Pohl et al., 2006). In the present study all stroke survivors used their paretic arm to perform the task, regardless of whether it was previously dominant or non-dominant. Currently it is not known whether the rate of learning on this task depends on hand dominance in any of the groups studied.

Lefebvre et al. (2014) used a motor learning task with stroke survivors that required similar functional movements as this task, i.e. movement of a computer mouse, in order to examine the effect of tDCS on motor learning. Learning in that task was achieved by changes in the speed-accuracy trade-off alongside learning of the movement pattern around the maze. The data collected from the present study could be used to inform a performance index calculation, as used by Lefebvre et al., for use in future studies with this paradigm. Dovern et al. (2011) also modified the SRTT to enable gross movements by using large buttons on a custom made response board. The main advantage of the paradigm developed in the present study over those alternative tasks is that sequence learning (OT), accuracy (PL) and speed can be examined individually using simple and portable pieces of equipment, thus being able to more thoroughly examine the implications of stroke, age and non-invasive brain stimulation on these individual aspects of motor learning.

3.5.5 Summary and limitations

Overall this study demonstrates the potential for this sequence learning paradigm to be used to assess both rate and amount of learning in healthy adults and also in people who have had a stroke when using the paretic arm. The pattern of motor learning is similar to that observed with traditional key-press sequence learning tasks, but less dexterous movements allow those with functional impairment to use their affected arm and hand to perform the task. This task allows assessment of both the total learning and the rate of learning, using differences between the last block of the recurring sequence and the subsequent random sequence, or with the area under the curve measurement. This is crucial as interventions such as tDCS could potentially alter rate, rather than total amount, of learning (Stagg et al., 2011). This idea is frequently overlooked when using the traditional SRTT paradigm.

Limitations of this study include small samples of participants and a heterogeneous group of stroke survivors. However, the results of this “proof of principle” study provide sufficient

evidence that this paradigm can be used to assess motor sequence learning for it to be used in future larger studies. However, not all of the stroke survivors were capable of completing the task, indicating that although this task is an improvement over the key press sequence learning tasks, it is still not suitable for the full range of functional impairments seen after stroke.

Primary motor cortex function is required for the acquisition and development of muscle synergies to promote faster and more accurate movements (for reviews see; Hardwick et al., 2013; Penhune and Steele, 2012; Shmuelof and Krakauer, 2011) and the paradigm developed here should be heavily dependent on the distributed motor learning network, including M1. Therefore it is hypothesised that tDCS to M1 should influence motor sequence learning. This hypothesis is testing in the forthcoming chapters.

Chapter 4 The effect of tDCS on motor sequence learning in healthy younger and older adults

4.1 Abstract

Background: Motor sequence learning may be improved with tDCS, but the impact of age and electrode arrangement is currently unclear.

Aims: To assess the effect of tDCS electrode arrangement on motor sequence learning and TCI in healthy young and older adults.

Methods: A cohort of 18 younger (mean age 27.7, range 19 - 42 years, 4 male) and 15 older (mean age 69.5, range 58 - 81 years, 4 male) adults received four sessions of tDCS during a motor sequence learning task involving movements of a computer mouse with the non-dominant hand to targets on a monitor in a repeated order. In each session tDCS was delivered in a different arrangement (crossover design); i) anodal to right M1, ii) cathodal to left M1, iii) bihemispheric and iv) sham. Change in iSP duration was assessed from each hand using TMS.

Results: The older adult group demonstrated impaired motor sequence learning ability compared with the younger group. Active tDCS did not improve learning, anticipations of target appearance or the shift in the speed-accuracy trade-off for either group. There was a significant increase in TCI from right to left M1 with bihemispheric tDCS in young adults only.

Conclusions: Overall tDCS does not improve motor sequence learning with this paradigm, potentially due to the explicit and rapid nature of the learning task which may involve regions other than M1.

4.2 Introduction

Transcranial direct current stimulation has been shown to be effective at improving sequence learning if applied during training (Kantak et al., 2012; Karok and Witney, 2013; Stagg et al., 2011; Zimerman et al., 2013), but not if applied prior to training (Amadi et al., 2015; Stagg et al., 2011). However, unilateral and bihemispheric electrode arrangements modulate cortical activity differently, likely due to differences in the direction of current flow (Lindenberg et al., 2013; Naros et al., 2016; Stagg and Johansen-Berg, 2013) and could therefore differentially affect connectivity between brain regions that influence sequential motor behaviours. The effect of electrode arrangement on motor sequence learning ability requires investigation.

Studies assessing the impact of tDCS on motor sequence learning in healthy older adults are limited and extrapolating results from studies with young adults to conditions such as stroke, which are more prevalent in older adults, may not be valid due to potential age-related differences in connectivity and plasticity. Further, healthy ageing is associated with a decline in physiological functions, including a reduced ability to learn motor skills (Doherty, 2003; Seidler et al., 2010), which tDCS may be able to ameliorate. This is potentially important with an ageing population. Zimerman et al. (2013) demonstrated improvements in motor sequence learning for older, but not younger, adults with anodal tDCS using a dexterous key press task. These findings have not been replicated, nor has the effect of anodal tDCS been compared with cathodal or bihemispheric montages to determine whether there is an optimal electrode arrangement for improving motor sequence learning.

Older adults have been shown to have reduced TCI compared with younger adults (Coppi et al., 2014; Davidson and Tremblay, 2013), and tDCS has been found to influence IHI (Tazoe et al., 2014). Change in TCI could be a potential mechanism underlying improvements in function but few studies have addressed this possibility. Williams et al. (2010) found a significant correlation

between change in IHI from non-dominant to dominant M1 and improvement in dexterity (JTT) of the non-dominant hand with bihemispheric tDCS combined with constraint of the dominant arm but this was not compared with unilateral tDCS.

Therefore, this study aimed to investigate the effect of tDCS electrode arrangement on motor sequence learning in healthy young and older adults. Specifically to determine whether:

1. active tDCS improved the rate of change in OT, speed and accuracy,
2. the tDCS electrode arrangement impacted on changes,
3. changes in motor sequence learning were related to changes in TCI,
4. the response to tDCS was dependent on age.

Based on previous literature it was hypothesised that:

1. the rate of change in OT, speed and accuracy would be improved with active tDCS compared to sham,
2. bihemispheric tDCS would provide additional benefit over unilateral,
3. improvements in motor sequence learning would be associated with an increase in TCI from right to left M1,
4. greater improvements in motor sequence learning would be evident for older adults compared with younger.

4.3 Methods

4.3.1 Participants

Recruitment was through emails, advertisements and word of mouth between May 2013 and October 2015. Inclusion criteria were; aged > 18 years and right handed (mean laterality index 75 %, range 33 – 100 %; Oldfield, 1971). Exclusion criteria were contraindications to TMS such as epilepsy or seizures, cardiac pacemakers or metal implants in the head. Participants denied any

neurological conditions or medications that could alter central nervous system excitability and all were community dwelling. All participants gave written informed consent and the study was approved by the local Research Ethics Committee (BDM/13/14-21).

In total 38 people consented to participate. Four attended the familiarisation session and then failed to attend any scheduled follow up sessions and one participant had to be withdrawn during the second tDCS session due to persistent difficulties with the stimulation automatically switching off. Therefore the final sample for analysis consisted of 18 younger (mean age 27.7, range 19 - 42 years, 4 male) and 15 older (mean age 69.5, range 58 - 81 years, 4 male) adults. Three of the participants from the younger group and one from the older group had also participated in the experiments from the study in Chapter 3, but at least 8 months separated the two studies.

4.3.2 Paradigm

The motor sequence learning task was similar to that described in Chapter 3. Briefly, participants sat at a table with a computer mouse on it, in front of a computer monitor (17 inch square) showing 4 grey circular targets (2.3 cm diameter) and a red central square (4.4 cm^2). The circular targets were all ~10.5 cm from the central square. A smaller central square and a longer sequence (12 movements) were used than for the experiments in Chapter 3 in an attempt to increase difficulty to reduce the likelihood of "ceiling effects". Participants used their non-dominant (left) hand to perform the task in order to make the task as difficult as possible and avoid ceiling effects.

4.3.2.1 Familiarisation session

Participants were familiarised with the motor sequence learning task without receiving tDCS in order to minimise potential differences between sessions due to familiarisation with the protocols. Participants completed as many repetitions of a sequence of 12 movements as

necessary to ensure that they felt comfortable with the use of the computer mouse with the non-dominant hand and understood the purpose of the task.

4.3.2.2 Experimental sessions

The remaining four sessions were conducted using a cross-over design with sessions at least one week apart (mean (standard deviation (SD)): 12 ± 8 days) to minimise carry over effects. The within-subject crossover design was chosen rather than a between-group design in an attempt to control for inter-individual variation in ability to learn the movement task. The time of day was kept as consistent as possible for each participant and each session lasted ~1 hour. In each session (Figure 4.1), participants initially received TMS (to localise M1 and assess TCI; see subsection 4.3.3). The motor learning task was then performed, while receiving tDCS. Following completion of the motor sequence learning task TMS was again delivered to assess TCI.

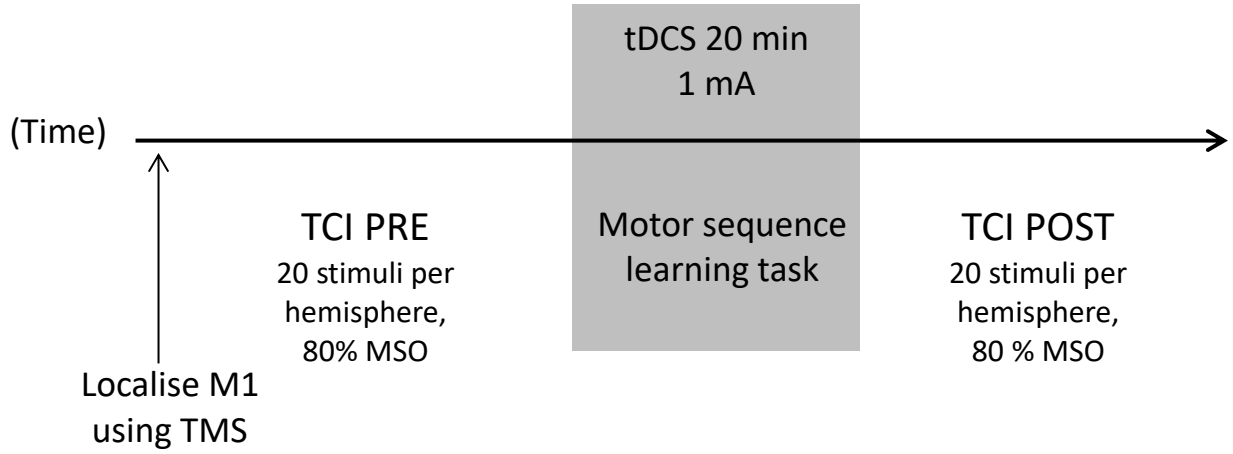


Figure 4.1 Diagram showing order of events for each session.
TMS = transcranial magnetic stimulation, MSO = maximum stimulator output, TCI = assessment of transcallosal inhibition, tDCS = delivery of transcranial direct current stimulation.

4.3.2.3 Motor sequence learning task

At the beginning of each session, participants initially completed a practice sequence to re-familiarise them with the movement of the mouse to the targets. They were then reminded that they would repeat a sequence of 12 movements, 25 times, and that they could anticipate target

appearance if they knew which would be next to illuminate. The sequence for each participant and session was chosen randomly from a pool of eight sequences, ensuring that a different sequence was performed in each session. Following completion of the 25 repetitions of the sequence, two random sequences (12 movements each) were performed to distinguish between general learning and sequence specific learning effects. Finally, one additional repetition of the trained sequence was completed. The OT for each repetition was normalised to the first repetition, herein referred to as "normalised OT". As specified in Chapter 3, values were averaged across consecutive repetitions to form 13 blocks.

In addition to OT, PL and speed (see Chapter 3) a performance index (PI) was calculated as used in previous motor learning studies (Lefebvre et al., 2012a; Lefebvre et al., 2012b). Using data from Chapter 3, with no stimulation, constant values were calculated for path length error (a) and speed (b). Path length error was calculated as the difference between the median path length for each repetition of the sequence and the minimum path length required to reach the targets. A learning index was calculated using the following formula (Lefebvre et al., 2012a):

(1) Learning index = (a / path length error) × (speed / b).

The learning index for each repetition was expressed relative to the first repetition of the sequence to give the PI. Values > 1 indicate improvement in either speed or accuracy without a reciprocal decrement in the other, or improvements in both speed and accuracy.

As described previously (Chapter 3) the number of accurate anticipations were calculated for each participant in each session, and the AUC was calculated for OT and PI to indicate the rate and amount of change. The specificity of leaning to the trained sequencer was determined as the difference in normalised OT or PI between the last block of the repeated sequence and the random block.

4.3.3 Stimulation of primary motor cortex

4.3.3.1 Setup

TMS was used to determine the position of the M1 representation of each FDI muscle for placement of the tDCS electrodes and for assessment of changes in TCI.

Electromyography (EMG) activity was recorded from each FDI using pairs of 13 mm Ag/AgCl Biotab electrodes (Unomedical Ltd, UK) placed over the muscle in a belly-tendon montage, following standard skin preparation techniques. Ground electrodes were placed over each ulnar styloid (23 mm Ag/AgCl Biotab electrode). The analogue EMG data were pre-amplified 1000× (Digitimer Ltd, Hertfordshire, UK) and bandpass filtered at 30-1000 Hz (Neurolog filter module, Digitimer Ltd, UK) with a 50 Hz notch filter. Data were acquired at 2 kHz, A to D converted (1401, Cambridge Electronic Design Ltd (CED), UK), recorded (Signal 4.07, CED, UK) and stored for off-line analysis.

A figure-of-eight coil (70 mm diameter) with a Magstim 200^2 Bistim stimulator (Magstim Company, UK) was used to elicit MEPs while participants rested their hands prone on a pillow on their laps. The optimal position for evoking MEPs in the relaxed FDI was established in each session and marked with a water-soluble marker directly on the scalp to ensure consistent coil placement.

4.3.3.2 Transcallosal inhibition

A TMS intensity of 80 % maximum stimulator output (MSO) was used to assess TCI. Participants were instructed to activate the FDI muscle at ~ 75 % of their maximal effort while single pulse stimuli were delivered to the ipsilateral M1 with an interstimulus interval of ~5 – 7 s. Twenty stimuli were delivered to each M1 before and immediately following performance of the motor sequence learning task. A short break (~ 30 s) was given after every five stimuli for the participant to relax their hand in an attempt to ensure they did not experience any fatigue.

The duration of TCI was calculated using Signal 4.07 (CED, UK). Each trace was rectified then an average waveform constructed. The pre-stimulus root mean square (RMS) EMG was calculated for a 450 ms period ending 10 ms before the stimulus. The duration of TCI was calculated for each trace from the time where the rectified EMG activity dropped below 75 % of the pre-stimulus level to when it returned above 75 %. This criterion for onset and offset of the iSP was chosen as it was deemed to provide an objective and robust method for determination that would minimise potential for bias from the experimenter. An average duration was calculated for each hemisphere pre- and post-stimulation.

4.3.3.3 Transcranial direct current stimulation

For the experimental sessions tDCS was delivered during the motor learning task for 20 minutes at 1 mA using a constant current stimulator (Mind Alive, Canada or NeuroConn, Rogue Resolutions, UK) with two carbon electrodes encased in 5 × 5 cm square saline-soaked (0.1 % NaCl) sponges (current density 0.04 $mA.cm^{-2}$). For anodal tDCS the anode was placed over the right M1 hotspot for FDI and the cathode over the contralateral supraorbital area, for cathodal tDCS the cathode was placed over the left M1 (ipsilateral to the performing hand) and the anode over the contralateral supraorbital area, and for bihemispheric tDCS the anode was placed over right M1 and the cathode over left M1 (Figure 4.2). With these arrangements the goal of all active conditions was to increase the excitability of the right M1 and the circuits controlling the left hand.

This study was a single-blind, sham controlled crossover trial. Sham stimulation was performed in a standard manner; current was ramped up for 30 s, in either of the electrode arrangements, then turned off. The order of tDCS conditions was randomised across participants using a Latin square design and participants were blinded as to which session was sham stimulation.

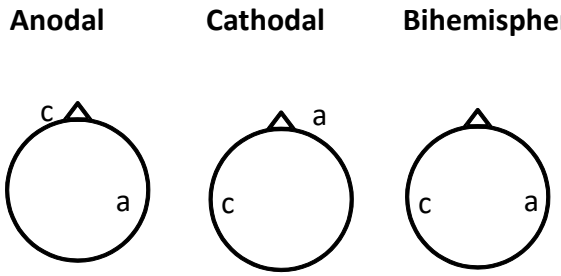


Figure 4.2 Representation of tDCS electrode arrangement.
a = anode, c = cathode

4.3.4 Statistical Analysis

Based on a previous motor sequence learning study (Zimerman et al., 2013) it was estimated that, for an effect size of 0.55, at least 28 participants would be required to find a difference in the OT AUC between active and sham stimulation with $\alpha = 0.05$ and power of 80 %.

Analysis was conducted using SPSS 21.0 (IBM Inc.) to dissociate effects on the whole group and also differential effects based on age group (younger < 45 years, older > 50 years). Normality of the residuals was assessed using Kolmogorov-Smirnov tests and visual inspection of frequency histograms. Violations of sphericity were corrected using the Greenhouse-Geisser correction. Data are presented as mean ± SEM and significance was set at $p < 0.05$, unless otherwise specified.

4.3.4.1 Task performance

A 12 BLOCK × 4 TDCS × 2 AGE GROUP mixed ANOVA was used to determine whether normalised OT or PI changed with training and whether this was dependent on tDCS electrode arrangement (sham, anodal, cathodal, bihemispheric) and age group (younger, older).

A 4 TDCS × 2 AGE GROUP mixed ANOVA was used to determine the effect of tDCS condition and group on the OT AUC, PI AUC, total accurate anticipations and normalised OT or PI difference between last block and random block. To compare directly between active stimulation

conditions a 3 TDCS × 2 AGE GROUP mixed ANOVA was used with values for anodal, cathodal and bihemispheric as a percentage of sham stimulation (% sham). One sample t-tests were used to determine whether values for active stimulation differed from sham (100 %).

4.3.4.2 Transcallosal inhibition

A 4 TDCS × 2 AGE GROUP mixed ANOVA was used to determine whether the change in iSP duration from each FDI was dependent on tDCS condition or group.

4.3.4.3 Relationships between variables

Pearson correlations were used to assess for relationships between change in iSP duration and total learning (OT or PI AUC), relative to sham.

4.4 Results

4.4.1 Onset time

4.4.1.1 Initial OT

The 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS on the baseline (first repetition) absolute OT ($F_{3,93}$ = 0.486, p = 0.693) and no interaction ($F_{3,93}$ = 0.848, p = 0.471). This indicates that baseline performance, i.e. reaction to target illumination, did not differ across tDCS conditions for younger or older adults. There was a significant effect of AGE GROUP ($F_{1,31}$ = 10.640, p = 0.003) as the OT (averaged across sessions) was quicker for the younger adults (0.34 ± 0.01 s) than the older group (0.41 ± 0.02 s), indicating slower choice reaction time for older adults.

4.4.1.2 OT over the blocks

The 4 TDCS × 12 BLOCK × 2 AGE GROUP mixed ANOVA showed an effect of BLOCK ($F_{23.7,115.8}$ = 150.13, $p < 0.001$) and a BLOCK by GROUP interaction ($F_{3.7,115.8}$ = 4.275, p = 0.004, Figure 4.3) but no effect of TDCS ($F_{3,93}$ = 0.848, p = 0.471), no two-way interaction between TDCS and BLOCK ($F_{10.6,328.5}$ = 0.819, p = 0.617) or three-way interaction between TDCS, BLOCK and AGE GROUP ($F_{10.6,328.5}$ = 0.711, p = 0.723).

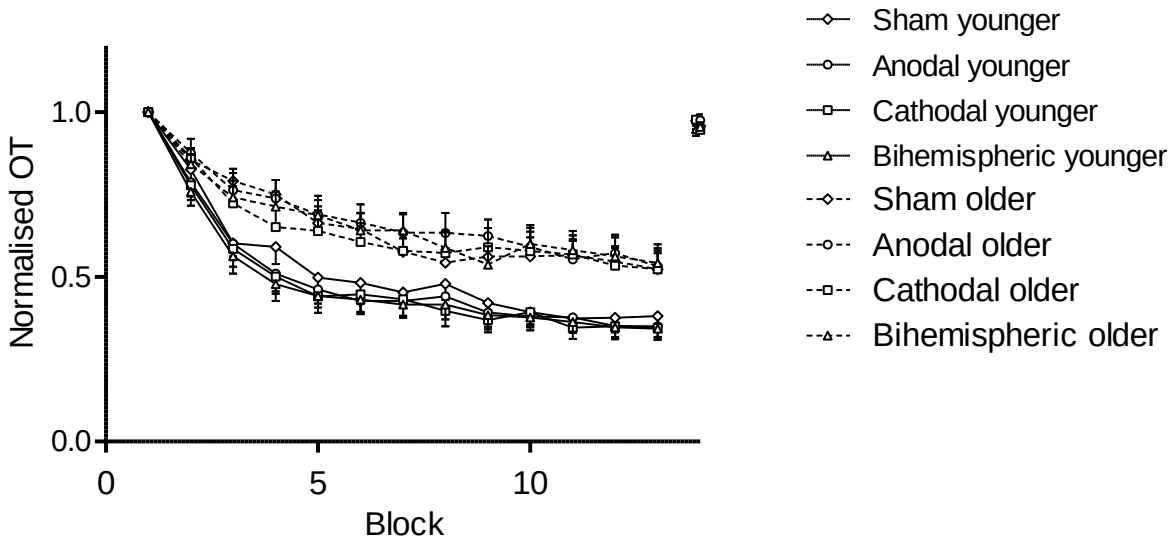


Figure 4.3 Normalised OT (mean ± SEM) for each age group under each tDCS condition. Younger group = solid lines, older group = dashed lines. Block 14 represents random block. There was a significant age group by block interaction (p = 0.004).

Post-hoc comparisons for the interaction between BLOCK and AGE GROUP indicated that normalised OT was lower for the younger group compared with the older for blocks 4 – 6 and 10 - 12 (independent samples t-tests with Bonferroni correction, $p \leq 0.004$, Figure 4.4). This indicates improved learning for the younger group, irrespective of tDCS condition. Full results can be found in Table B1, Appendix B.

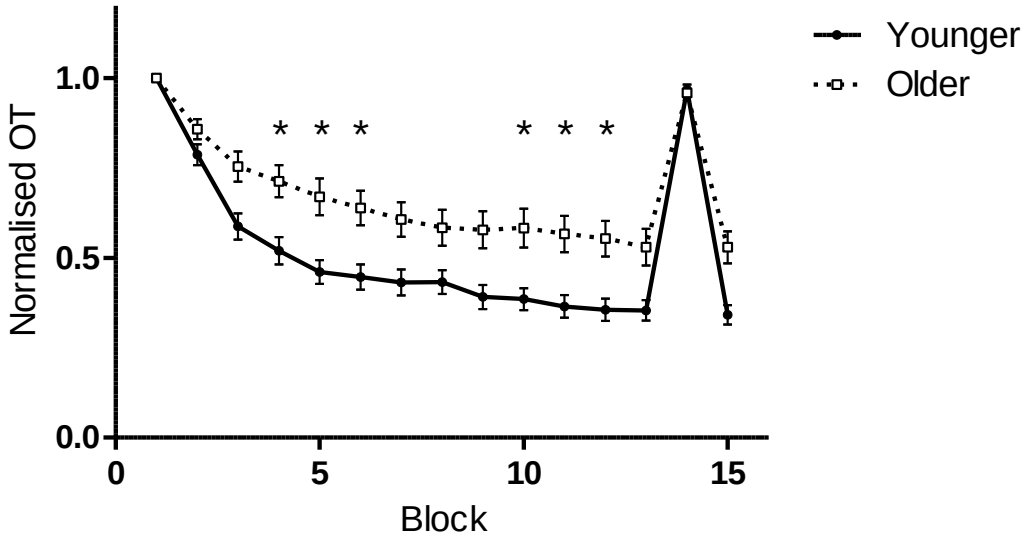


Figure 4.4 Normalised OT averaged across tDCS conditions (mean ± SEM).
Filled circles = younger, open squares = older adults. Block 14 represents random block, block 15 is the repeat of the trained sequence. There was an interaction between block and group. * lower OT for younger group, $p \leq 0.004$.

4.4.1.3 Total learning

The 4 TDCS × 2 AGE GROUP mixed ANOVA for the OT AUC showed no effect of TDCS ($F_{3,93}$ = 0.858, p = 0.466) and no interaction ($F_{3,93}$ = 0.852, p = 0.469) but an effect of AGE GROUP ($F_{1,31}$ = 10.674, p = 0.003, Figure 4.5). When averaged across tDCS conditions, OT AUC was significantly lower for the younger group (11.4 ± 0.7 arbitrary (arb.) units) compared with the older (15.5 ± 1.1 arb. units, t(31) =-3.267 , p = 0.003), indicating improved total learning for the younger group regardless of the tDCS condition.

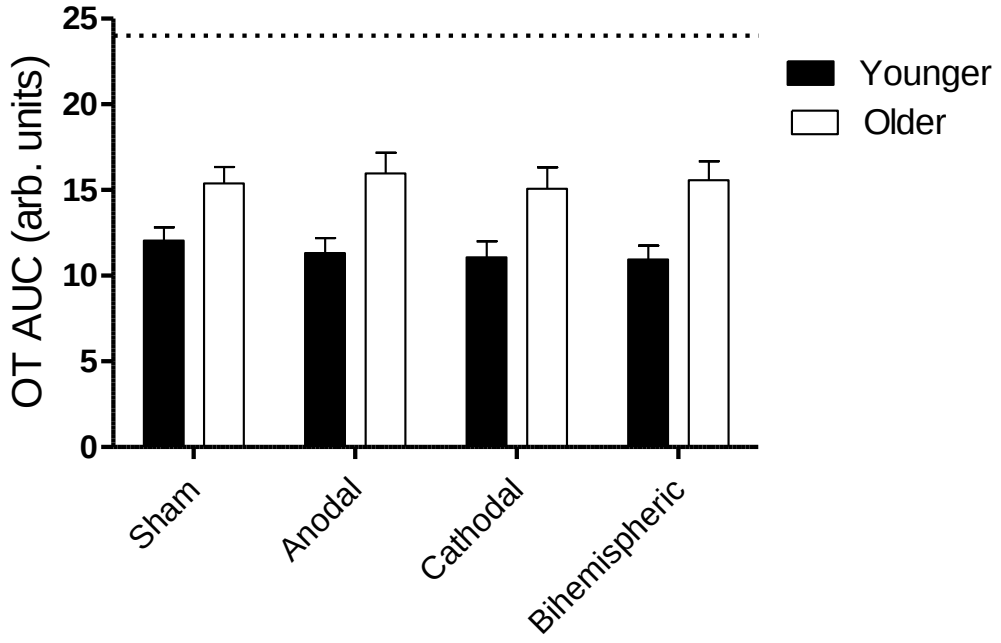


Figure 4.5 OT AUC (mean ± SEM) for younger and older adults under each tDCS condition. Dotted line indicates AUC if no change in OT occurred. There was a significant effect of group and younger adults showed significantly lower OT AUC values than older adults (p = 0.003).

The 3 TDCS × 2 AGE GROUP mixed ANOVA for the OT AUC (% sham) also showed no effect of TDCS ($F_{2,62}$ = 1.425, p = 0.248) and no interaction ($F_{2,62}$ = 0.293, p = 0.747, Figure 4.6). When averaged across tDCS conditions and groups (97.5 ± 3.6 %) a one-sample t-test found no difference from 100 % (t (32) = -0.692, p = 0.494), indicating that active tDCS did not alter total learning compared with sham.

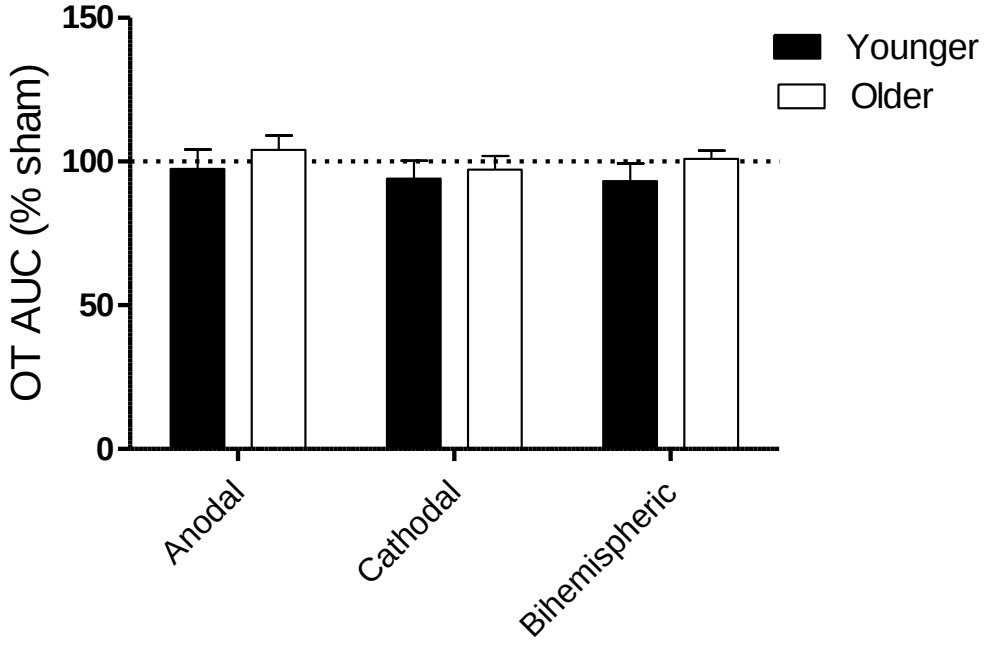


Figure 4.6 OT AUC as a percentage of sham stimulation (mean ± SEM) for each tDCS condition. Values < 100 % indicate greater reduction in OT with learning than for sham session. There was no effect of tDCS condition or age group (p > 0.2).

4.4.1.4 Specificity of sequence learning

The 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS on the difference in OT between the last block of the repeated sequence and the random block ($F_{2.3,71.4}$ = 0.440, p = 0.674) and no interaction ($F_{2.3,71.4}$ = 0.358, p = 0.783, Figure 4.7). There was a significant effect of AGE GROUP ($F_{1,31}$ = 13.253, p = 0.001) as the difference was greater for the younger (0.60 ± 0.04) than the older adults (0.43 ± 0.04), indicating improved sequence specific learning for younger adults.

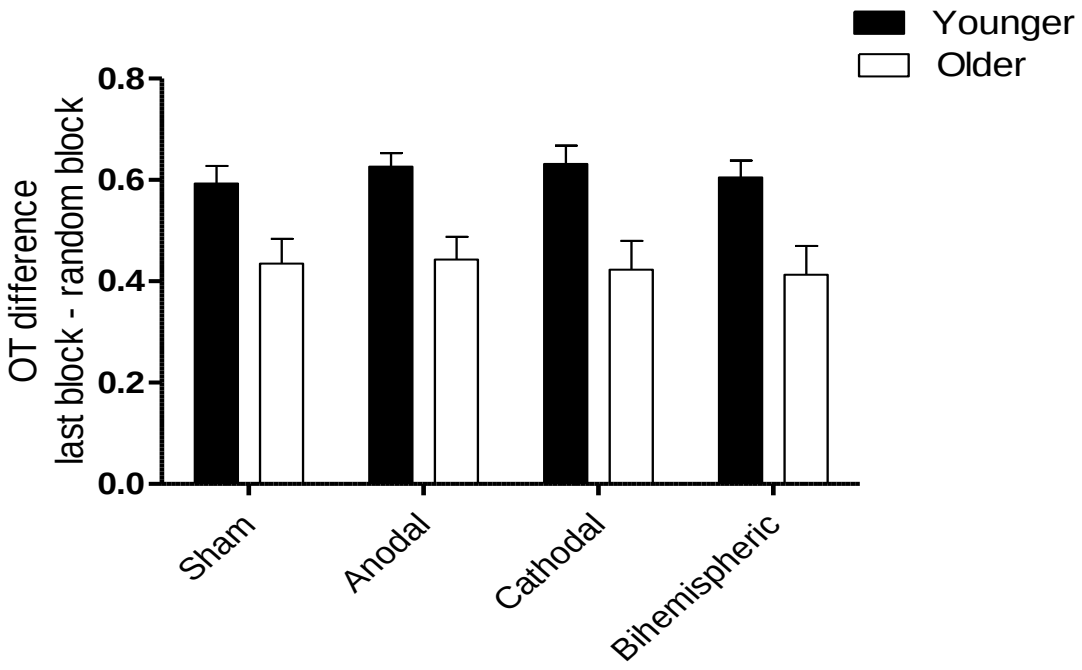


Figure 4.7 OT difference from last to random block (mean ± SEM) under each tDCS condition. There was no effect of tDCS condition, but younger adults showed a significantly greater OT difference than older (p = 0.001) irrespective of tDCS condition.

When expressed relative to sham, the data were not normally distributed and transformation did not achieve normality. The Friedman test (with younger and older adults together) showed no effect of TDCS (p = 0.469). When data were pooled (OT difference (% sham) = 110 ± 10 %) a one-sample Wilcoxon signed rank test found that there was no difference from 100 % (p = 0.741), indicating that active tDCS did not alter the specificity of learning in comparison with sham stimulation.

4.4.2 Total accurate anticipations

The 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS on the total number of accurate anticipations ($F_{3,93}$ = 0.869, p = 0.460) and no interaction ($F_{3,93}$ = 0.370, p = 0.775). There was a significant effect of AGE GROUP ($F_{1,31}$ = 16.629, p < 0.001) as the total number of accurate anticipations (averaged across conditions) was higher for the younger group (153 ± 13) than the older adults (75 ± 13, Figure 4.8). This shows that performance was better for younger adults compared with older, irrespective of tDCS condition.

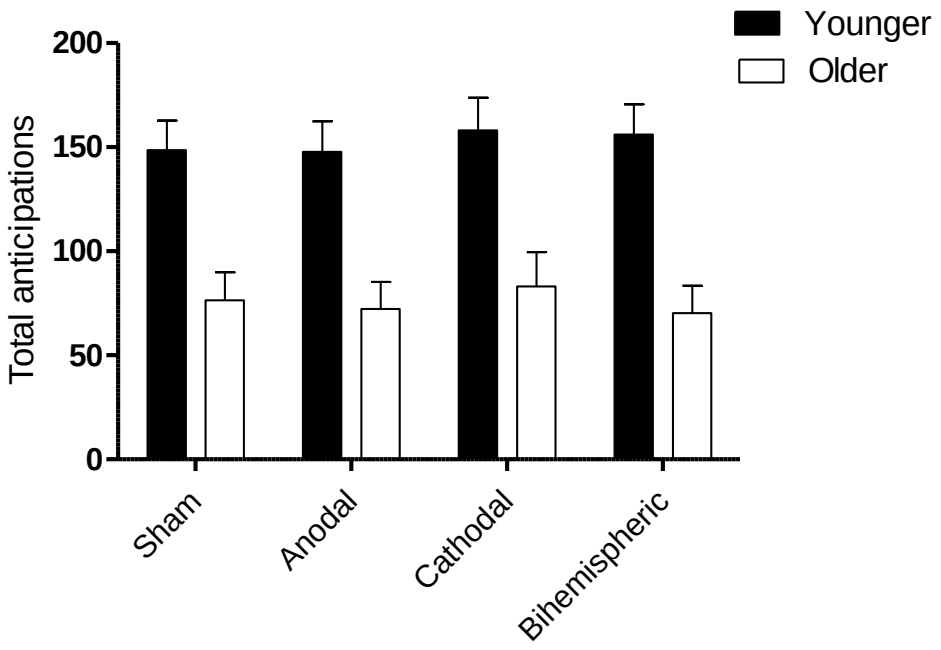


Figure 4.8 Total number of accurate anticipations (mean ± SEM) under each tDCS condition. There was a significant effect of age group, with older adults showing less anticipations (p < 0.001).

When expressed relative to sham, the 3 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS ($F_{1.7,52.1}$ = 0.721, p = 0.468), no interaction ($F_{1.7,52.1}$ = 0.783, p = 0.442) and no effect of AGE GROUP ($F_{1,31}$ = 0.717, p = 0.403). When data were pooled (Total anticipations (% sham) = 107 ± 7 %) a one-sample t-test found no difference from 100 % (t(32) = 1.036, p = 0.308), indicating that active tDCS did not affect the number of anticipations.

4.4.3 Speed-accuracy trade-off

Initial movement speed (averaged across sessions) was significantly slower for older adults (802 ± 39 pixels.s^{-1}) compared with younger (1060 ± 45 pixels.s^{-1}, independent samples t-test, t(31) = 4.283, p < 0.001).

For PI, the 4 TDCS × 12 BLOCK × 2 AGE GROUP mixed ANOVA showed an effect of BLOCK ($F_{6.7,209.3}$ = 10.2, p < 0.001) but no effect of TDCS ($F_{3,93}$ = 0.228, p = 0.877) or AGE GROUP ($F_{1,31}$ = 0.044, p = 0.835) and no interactions. Figure 4.9 shows data pooled across tDCS condition and age group indicating improvements in the speed-accuracy trade-off with practice, but irrespective of tDCS condition.

To determine whether changes in PI were specific to the trained sequence a paired samples t-test was used to compare the PI for the last block of the repeated sequence with the random block (averaged across tDCS condition and age group). There was no significant difference (t(32) = 1.723, p = 0.094) indicating that increases in PI were not specific to the trained sequence.

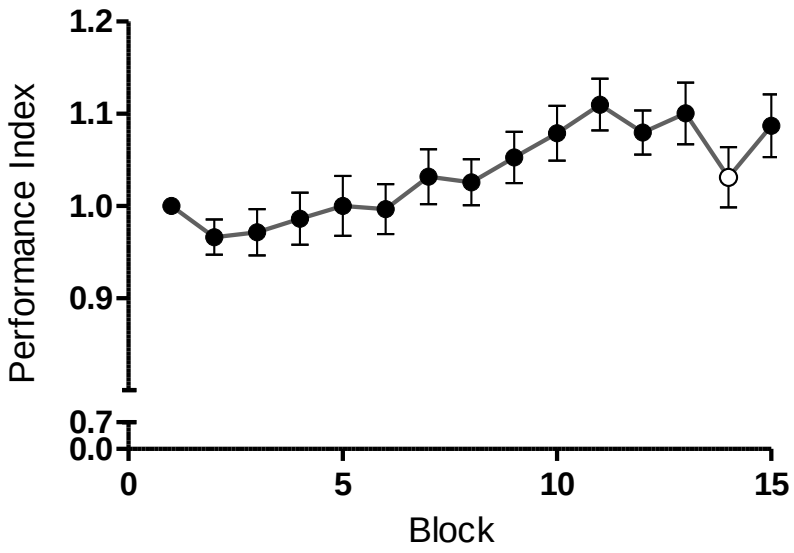


Figure 4.9 PI for each block averaged across age group and tDCS condition (mean ± SEM).
Block 14 (open circle) represents the random sequence and block 15 the repeat of the trained sequence.
There was a significant effect of block, indicating improvements with training.

PI AUC was not normally distributed so a log transformation was applied. The 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS on the PI AUC ($F_{3,93}$ = 0.277, p = 0.842), no effect of AGE GROUP ($F_{1,31}$ = 0.045, p = 0.833) and no interaction ($F_{3,93}$ = 0.386, p = 0.763, Figure 4.10). Similarly, when expressed relative to sham, the 3 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS ($F_{2,62}$ = 0.595, p = 0.555) or AGE GROUP ($F_{1,31}$ = 0.821, p = 0.372) and no interaction ($F_{2,62}$ = 0.161, p = 0.852). When averaged across tDCS conditions and age group (104 ± 4 %) a one-sample t-test found no difference from 100 % (t(32) = 0.874, p = 0.389), indicating that active tDCS had no effect on changes in the speed-accuracy trade-off in comparison with sham stimulation.

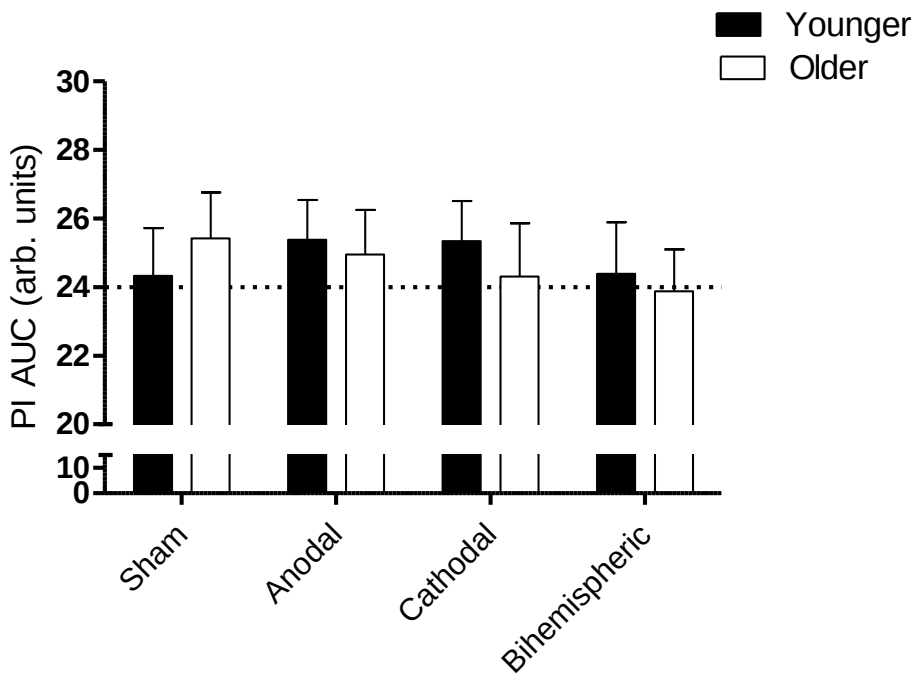


Figure 4.10 PI AUC (mean ± SEM, non-transformed) for each tDCS condition.
Dotted horizontal line indicates AUC if no change occurred. There was no effect of tDCS condition or age group (p > 0.8).

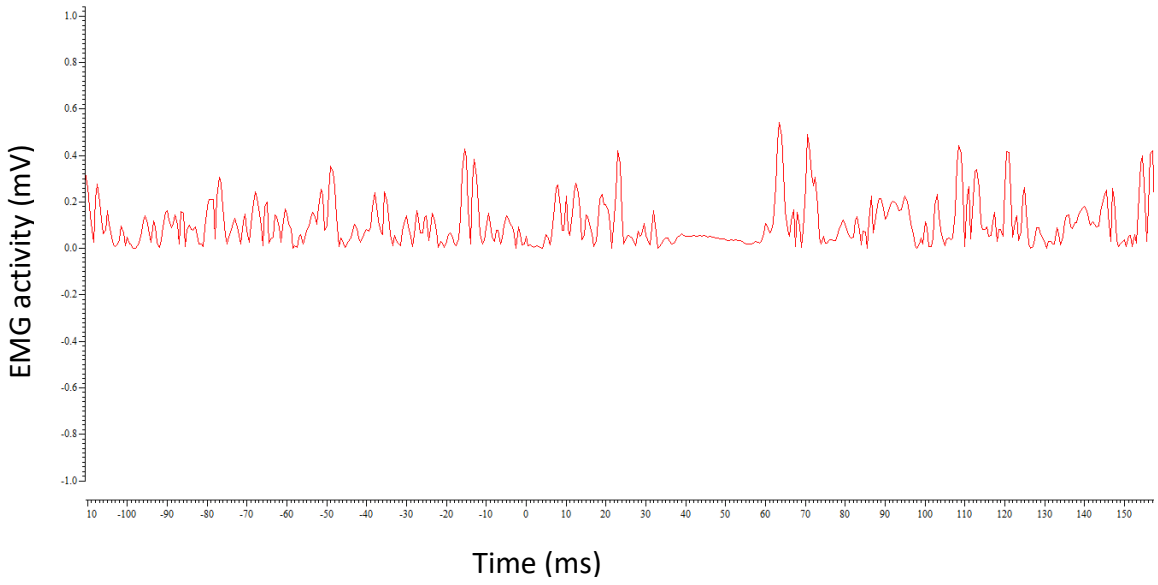
The 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS on the PI difference between the last block of the repeated sequence and the random block ($F_{3,93}$ = 0.633, p = 0.596), no TDCS by AGE GROUP interaction ($F_{3,93}$ = 0652, p = 0.584) and no effect of AGE GROUP ($F_{1,31}$ = 0.663, p = 0.422).

To determine whether changes in PI were specific to the trained sequence a paired samples t-test was used to compare the PI for the last block of the repeated sequence with the random block (averaged across tDCS condition and age group). There was no significant difference ($t(32) = 1.723$, $p = 0.094$) indicating that increases in PI were not specific to the trained sequence.

4.4.4 Transcallosal inhibition

To ensure that voluntary activation (EMG) was consistent pre-post stimulation and across sessions a 4 TDCS × 2 TIME rmANOVA was used for pre-trigger RMS EMG for each hand separately using log transformed data. Additionally, a 4 SESSION × 2 TIME rmANOVA was conducted to test for differences in pre-trigger RMS EMG across sessions, irrespective of tDCS condition.

For the left hand there was no effect of TDCS ($F_{3,90} = 1.014$, $p = 0.390$) or TIME ($F_{1,30} = 1.823$, $p = 0.187$) and no interaction ($F_{2.4,\ 73.3} = 0.568$, $p = 0.637$). Similarly, there was no effect of SESSION ($F_{3,90} = 0.850$, $p = 0.470$) and no interaction between TIME and SESSION ($F_{3,\ 90} = 0.300$, $p = 0.825$). For the right hand there was no effect of TDCS ($F_{3,90} = 0.809$, $p = 0.492$), but an effect of TIME ($F_{1,30} = 9.391$, $p = 0.005$) as pre-trigger RMS EMG was higher for the post-tests (non-transformed: 0.111 ± 0.007 mV) than the pre (0.102 ± 0.006 mV). However, there was no interaction between TDCS and TIME ($F_{2.4,73.2} = 1.530$, $p = 0.220$) indicating that this difference was not dependent on tDCS condition. There was an effect of SESSION ($F_{3,90} = 3.711$, $p = 0.0.14$) and with Bonferroni correction the pre-trigger RMS EMG tended to be higher for session one than either three ($p = 0.057$) or four ($p = 0.059$). However, there was no interaction between SESSION and TIME ($F_{3,90} = 0.826$, $p = 0.483$) indicating that this difference was consistent both pre- and post-stimulation. Additionally, there was no correlation between pre-trigger RMS EMG and iSP duration for either hand, pre- or post-stimulation for any tDCS condition ($p > 0.05$).



An example EMG trace is shown in Figure 4.11. For iSP duration from right FDI (right to left M1 TCI), the 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS on the change in iSP duration ($F_{3,87}$ = 1.735, p = 0.362) but a tendency toward a TDCS by AGE GROUP interaction ($F_{3,87}$ = 2.612, p = 0.056) and a tendency for an effect of AGE GROUP ($F_{1,29}$ = 3.312, p = 0.079). The tendency toward an interaction was brought about because iSP duration increased for bihemispheric tDCS compared with all other conditions in the younger (one-way rmANOVA $F_{3,51}$ = 4.912, p = 0.004 with pairwise comparisons, p ≤ 0.01) but not the older adults ($F_{3,36}$ = 0.254, p = 0.858, Figure 4.12 and Appendix C).

For iSP duration from left FDI (left to right M1 TCI), the 4 TDCS × 2 AGE GROUP mixed ANOVA showed no effect of TDCS ($F_{3,87}$ = 0.512, p = 0.675) or AGE GROUP ($F_{1,29}$ = 0.029, p = 0.865) but a tendency toward an interaction between TDCS and AGE GROUP ($F_{1,29}$ = 2.477, p = 0.067) on the change in iSP duration. However, when separate one-way rmANOVAs tested each age group there was no effect of tDCS condition for younger ($F_{3,51}$ = 2.022, p = 0.122) or older ($F_{3,36}$ = 1.098, p = 0.362) adults (Figure 4.12 and Appendix C).

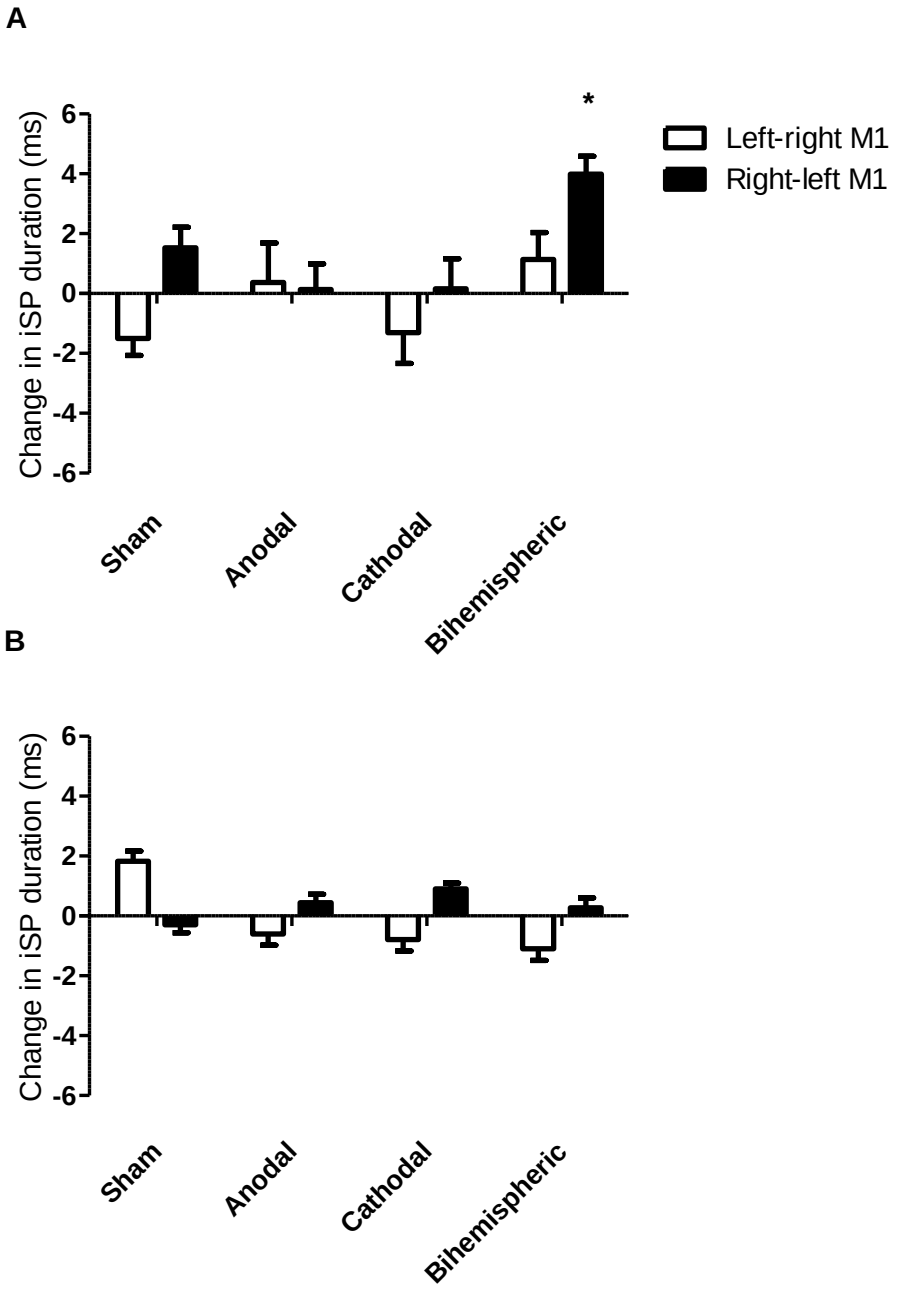


Figure 4.12 Change in iSP duration (mean ± SEM) from each hemisphere under each tDCS condition. **A.** Younger adults; iSP duration is increased from right to left M1 with bihemispheric tDCS compared to all other conditions, $p < 0.01$. **B.** Older adults; there were no effects of tDCS condition.

4.4.5 Relationships between transcallosal inhibition and learning

There were no significant correlations between change in TCI from either right or left M1 and OT AUC ($p > 0.1$, Figure 4.13) or PI AUC ($p > 0.05$, Figure 4.14) relative to sham (Table D1, Appendix D).

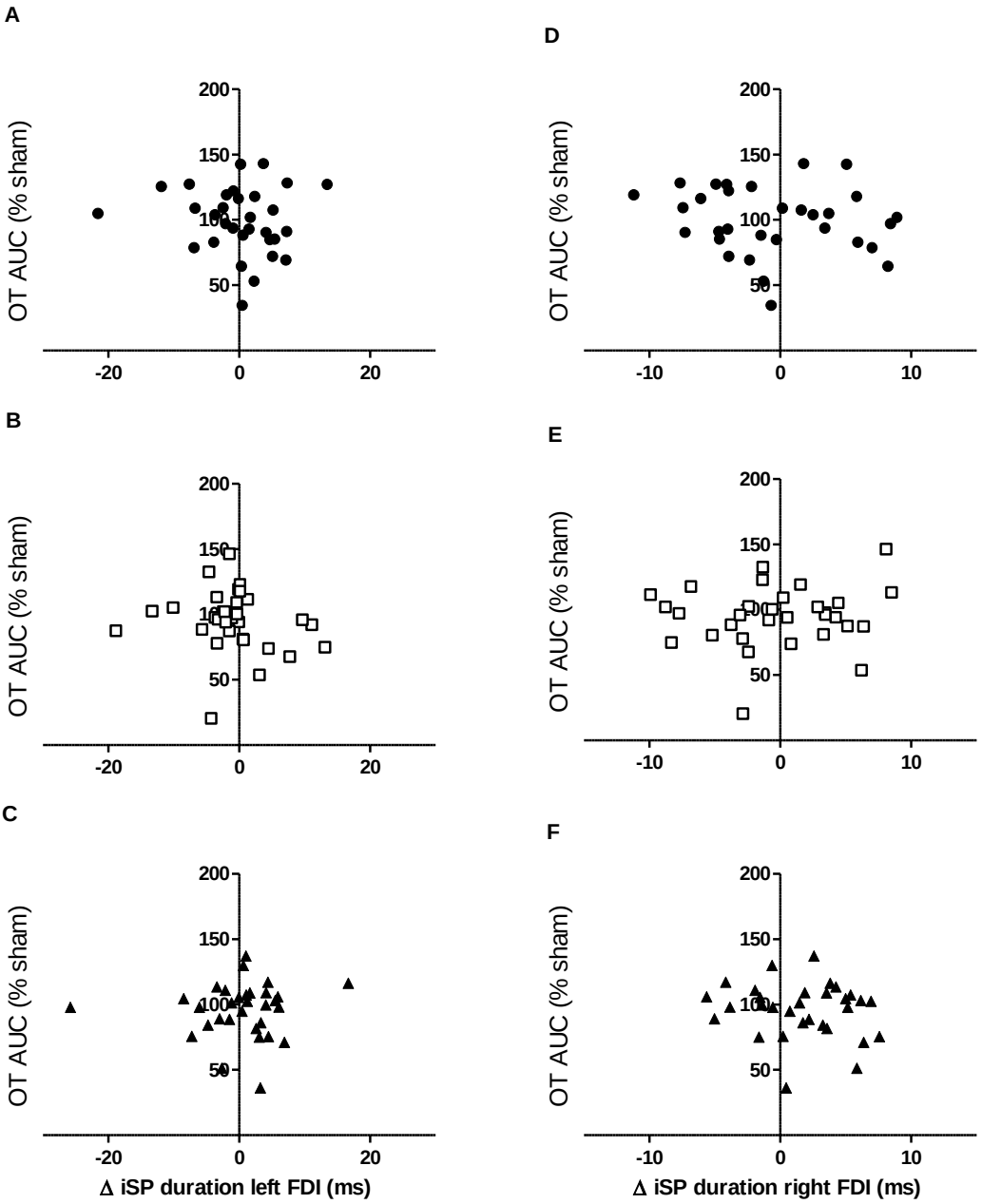


Figure 4.13 OT AUC (% sham) as a function of change in iSP duration.
Left column: left FDI (left to right M1 TCI), right column: right FDI (right to left M1 TCI). **A,D** = Anodal tDCS, **B,E** = Cathodal tDCS, **C,F** = Bihemispheric tDCS. Y axis value < 100 % indicates improved learning over sham as a lower OT AUC value indicates a greater reduction in OT over the learning period. There were no significant correlations.

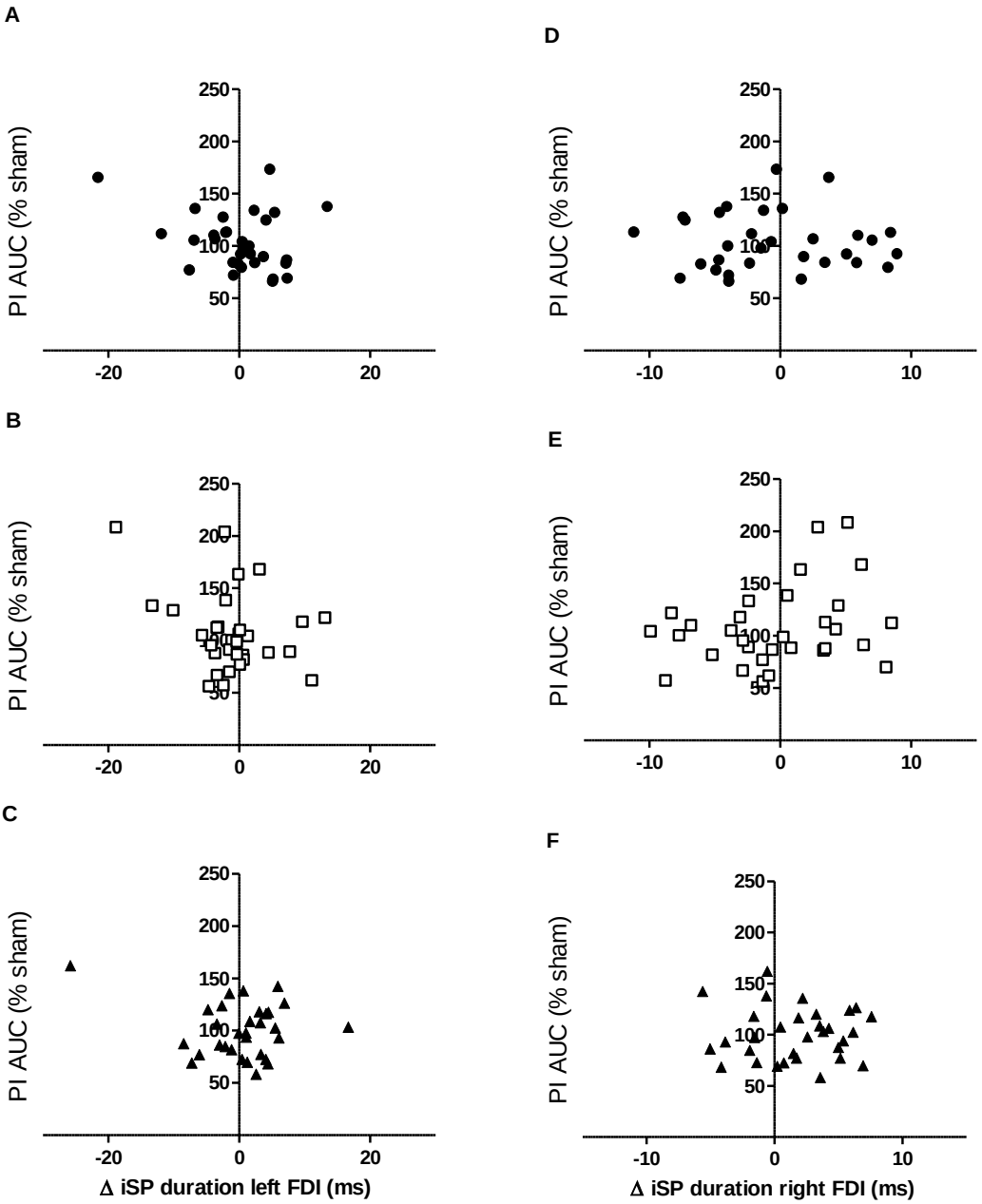


Figure 4.14 PI AUC (% sham) as a function of change in iSP duration.
Left column: left FDI (left to right M1 TCI), right column: right FDI (right to left M1 TCI). **A,D** = Anodal tDCS, **B,E** = Cathodal tDCS, **C,F** = Bihemispheric tDCS. Y axis value > 100 % indicates improved performance over sham as a greater PI AUC indicates greater increases in the PI over the learning period. There were no significant correlations.

4.5 Discussion

The main finding of this study was that active tDCS did not improve motor sequence learning within a session for younger or older adults. However, impairments in learning were evident for older adults and there was an increase in transcallosal inhibition with bihemispheric tDCS only for younger adults.

4.5.1 No alteration in rate of change of onset time or performance index with tDCS

There was no effect of tDCS on the pattern of change in OT or PI, or on the AUC which takes into account both the rate and the amount of change over the blocks. This indicates that tDCS did not alter learning with this paradigm. Whilst some previous studies have indicated improvements in motor learning with active tDCS compared to sham in healthy adults (Kantak et al., 2012; Naros et al., 2016; Nitsche et al., 2003c; Stagg et al., 2011; Vines et al., 2008; Zimerman et al., 2013), others have not found significant within-session improvements (Amadi et al., 2015; Ambrus et al., 2016; Kang and Paik, 2011). It is unclear exactly why different findings exist with apparently similar paradigms, but likely issues include: differences in stimulation parameters (current density, exact electrode locations); slight differences in the tasks (sequence difficulty, number of repetitions, breaks between blocks); familiarisation with the protocols; and the inherent variability between people in response to tDCS. The current study utilised 25 cm^2 electrodes, whereas some studies have used 35 cm^2 electrodes (e.g. Karok and Witney, 2013; Nitsche et al., 2003c; Stagg et al., 2011) which may also stimulate regions close to M1 such as PMC and SMA. However, Zimerman et al. (2013) also used 25 cm^2 electrodes and observed improvements with anodal tDCS for older adults.

The paradigm utilised here was different to the majority of sequence learning tasks used by others as it involved gross arm movements, rather than dexterous finger movements. This may have led to a task which was relatively less difficult and therefore had reduced capacity for

improvements with tDCS to be detected. Repetitions of the sequence were separated by short breaks while the repetition number was recorded and the Matlab programme restarted. Amadi et al. (2015) speculated that short breaks between repetitions could result in a reversal of the tDCS-induced improvements in performance as tDCS would essentially be applied both during and before learning. Learning is not improved with tDCS before training and therefore there is an overall null effect (Amadi et al., 2015; Stagg et al., 2011). The results of the current study are consistent with this hypothesis, as tDCS did not overall improve or impair learning performance.

A recent systematic review (Hashemirad et al., 2016) found that, although some studies show improvements in motor learning with tDCS, there are overall no significant improvements in movement speed, accuracy or skill for motor learning tasks (SRTT, sequential tap and SVIPT) during or immediately after anodal or bihemispheric tDCS. Combined with the results of the current study, this suggests that a single session of tDCS applied to M1 may be insufficient to produce reliable within-session improvements in motor sequence learning performance. It may be that tDCS is more effective at consolidating learning related improvements (Reis et al., 2009) through improved formation of motor memories, rather than improving learning *per se.*

Learning with the current task was rapid (Figure 4.4, page 90) in comparison with implicit learning tasks. A lack of improvement in learning with tDCS of M1 suggests that M1 may not play a crucial role in the control of explicit sequence learning ability. However, this idea is negated by a study which found that cathodal tDCS to the *contralateral* M1 impaired explicit sequence learning (Stagg et al., 2011), suggesting a functional role of M1. Additionally, the initial rapid phase of motor learning, whereby a task specific processing routine is established, is thought to involve M1 in addition to prefrontal and secondary motor areas (Hikosaka et al., 2002; Karni et al., 1998). Explicit sequence learning tasks have been found to show stronger PMd, SMA, SPL and thalamus activation compared with the implicit SRTT (Hardwick et al., 2013) and it may be that tDCS of one of these regions could have produced a stronger effect.

The non-dominant hand was chosen for the current study in an attempt to reduce the likelihood of "ceiling effects", since most of the participants routinely used the dominant hand to control a computer mouse. However, Hardwick et al. (2013) found that sequence learning tasks activated left, but not right, M1 and it is therefore possible that the results of the current study would have been different if the dominant hemisphere had been the target instead. Some aspects of movement have been shown to be primarily controlled by activity of the left (dominant) M1 in right handed people, such as motor imagery (Fadiga et al., 1999; Nair et al., 2003; Stinear et al., 2006) and fine finger movements (Kim et al., 1993), and it is possible that sequence learning may also rely more on the left M1 than the right. Indeed, several of the studies that demonstrated improvements in sequence learning with tDCS used the right hand and applied the anode over left M1 (Nitsche et al., 2003c; Stagg et al., 2011; Zimerman et al., 2013). However, improvements with the left hand following tDCS of right M1 have also been demonstrated (Kantak et al., 2012; Vines et al., 2008) so the idea of hemispheric asymmetry in the control of motor sequence learning requires further investigation.

4.5.2 The effect of tDCS electrode arrangement remains unknown

There was no overall effect of tDCS on motor sequence learning for either younger or older adults and therefore it is not possible determine any differential effects based on electrode arrangement on this task. In contrast, a recent study has found bihemispheric tDCS to improve performance of a motor learning task involving proximal arm movements, compared with anodal or cathodal alone (Naros et al., 2016). Using finite-element modelling the authors reported differences in current flow: a posterior-anterior current flow towards premotor and frontal areas with unilateral stimulation, compared to a bilateral current flow between sensorimotor areas covering premotor and parietal cortex with bihemispheric stimulation. These differences in current flow, and therefore modulation of neural activity, could account for the differences in response between electrode arrangements.

Although electrode arrangement did not affect motor sequence learning in the current study, differential effects on TCI were observed. There was an increase in iSP duration from the right FDI with bihemispheric tDCS in younger adults compared to both sham stimulation and unilateral tDCS (Figure 4.12, page 99). Changes in IHI with bihemispheric tDCS have been demonstrated previously (Tazoe et al., 2014; Williams et al., 2010). Williams et al. (2010) found a decrease in IHI from the dominant (site of the cathode) to the non-dominant M1, but no change from the non-dominant to the dominant. The present study shows only an increase in TCI from the non-dominant (site of the anode) to the dominant M1. In the study by Williams et al. (2010) the right (dominant) hand was constrained, so it may have been that forced non-use of the hand is necessary to produce the reduction in transcallosal inhibition. However, Tazoe et al. (2014), who applied tDCS at rest, found the hypothesised increase in right to left M1 IHI and concurrent decrease in left to right M1 IHI. The increase in TCI duration in the current study with bihemispheric tDCS only may suggest that the direction of current flow when the electrodes are placed on each M1 is preferential for modulating the activity of transcallosal neurons, as bihemispheric tDCS induces a more lateral to medial current flow than the unilateral arrangements (Naros et al., 2016). Therefore, the hypothesis of bihemispheric tDCS acting through an effect on interhemispheric interactions may be valid, even though there was no decrease in duration from left to right M1 in the current study.

There appeared to be little change in TCI under any condition for the older adults. Similarly, changes in MEP amplitude were only observed for the younger group (Appendix E), suggesting that the tDCS did not alter neuronal excitability for the older group. This may explain why older adults did not show improvements in learning with tDCS despite clear impairments relative to the younger group. This could have implications for the use of tDCS in stroke survivors, who are predominantly older adults. However, the assessment of TCI (and MEP amplitude) was done immediately after the completion of the tDCS and it is possible that changes in cortical activity were missed for the older adults who may show a delayed response (Fujiyama et al., 2014).

Additionally, there was a high level of variability between subjects. Variation in modulation of MEP amplitude within and between subjects has been demonstrated by several studies (Dyke et al., 2016; Horvath et al., 2016; Lopez-Alonso et al., 2014; Wiethoff et al., 2014), although changes in TCI have been reported to be more consistent (Davidson et al., 2016). Even though only the younger group showed a significant increase in TCI with bihemispheric tDCS in the current study, 77 % of the total sample (including the older adults) showed a change in duration that was positive suggesting similar consistency as Davidson et al. (2016) reported. Variability in response to tDCS could be due to individual differences in brain structure affecting the current flow, skull thickness affecting the depth of current in the cortex, baseline neural excitability, inhibition or connectivity and their capacity for plasticity (for review see Li et al., 2015). Unfortunately, the results of the current study do not provide any further information to aid understanding as to why some people respond and others do not. The behavioural response to tDCS (i.e. motor sequence learning changes) was also highly variable, with 24 % of participants showing OT AUC improvements in all active tDCS conditions compared to sham, 33 % showing worse improvement with active tDCS and the remainder showing a mixed response. Since this was a crossover design study it is possible that variation in performance between sessions had a greater influence than the tDCS effect and a between-group study design may be warranted.

4.5.3 No relationship between transcallosal inhibition and learning

The results of the Pearson correlations indicated no relationships between the change in TCI duration and total learning (OT AUC, % sham; Figure 4.13), or the change in the speed-accuracy trade-off (PI AUC, % sham; Figure 4.14). This may suggest that, although a significant increase in TCI was found for the younger group, changes in TCI do not underlie improvement in learning. This conclusion is partially consistent with Kidgell et al. (2013b) who reported no relationships between change in Purdue pegboard performance and neurophysiological changes (MEP amplitude and SICI). In contrast, Williams et al. (2010) found a significant moderate to strong correlation between the change in IHI from left (site of the cathode) to right M1 and

improvement in dexterity (JTT) with bihemispheric tDCS combined with constraint of the dominant arm. The reduction in IHI from the site of the cathode may therefore play a crucial role in the promotion of function. However, the lack of overall effect of tDCS on the current task makes it difficult to draw any definitive conclusions.

It must also be considered that although rebalancing of interhemispheric connections is frequently cited as a rationale for using tDCS, TCI may not have been the optimal measure to attempt to understand the neurophysiological changes with tDCS. Previous studies have demonstrated that GABA concentration is changed with both anodal (Kim et al., 2014; Stagg et al., 2009) and cathodal (Stagg et al., 2009) tDCS but not bihemispheric (Tremblay et al., 2016), that the change in intracortical inhibition (GABA and SICI) correlates with motor learning (Kim et al., 2014; Zimerman et al., 2012), and that the GABA concentration within ipsilesional M1 can predict behavioural improvement with anodal tDCS in stroke survivors (O'Shea et al., 2014). Therefore, intracortical inhibition may have a greater role to play in the effects of tDCS than TCI.

4.5.4 The effect of age on the response to tDCS remains unclear

There were no differential effects of tDCS on motor sequence learning based on age group. This contrasts with other studies which have indicated that older adults may benefit from tDCS to a greater extent than younger adults (Hummel et al., 2010; Zimerman et al., 2013). Differences could be because the task in the current study did not involve dexterity, but rather gross movements of the hand and arm which may be less sensitive to improvements with tDCS. However, consistent differences were found between younger and older adults for motor sequence learning performance, as has been demonstrated previously (Boyd et al., 2008; Zimerman et al., 2013). The initial OT was found to be significantly slower for the older adults than the younger, indicating slower choice reaction times, which has been demonstrated previously (Curran, 1997; Der and Deary, 2006; Francis and Spirduso, 2000; Woods et al., 2015). There was also an interaction between group and block for normalised OT, suggesting a different

pattern of learning between younger and older adults (Figure 4.4, page 90). The reduction in OT was more pronounced for younger adults, the OT AUC was significantly less and the OT difference between the last block of the repeated sequence and the random block was greater, indicating superior motor sequence learning performance for the younger group. Zimmerman et al. (2013) demonstrated an improvement in motor sequence learning performance with tDCS for older adults that was not present for younger adults. This may have been indicative of a ceiling effect for the younger adults who, as in the current study, had better performance without stimulation than the older adults. Despite impairments in performance for the older group, the current study failed to replicate their finding, with no improvements for either group with active tDCS compared to sham. Participants potentially used a strategy to learn the sequence that was less dependent on M1 than the "key press" tasks and therefore not improved by tDCS to this region.

A novel finding from this study was a higher number of anticipations of target appearance, irrespective of tDCS condition, for the younger adults (Figure 4.8, page 94). This could be a physiological effect, or could be psychological with the possibility that older participants were less confident in their abilities or more concerned about anticipating incorrectly. However, this was not specifically assessed.

4.5.5 Speed-accuracy trade off

Older adults had significantly slower movement speed initially which could be a compensation mechanism to ensure accuracy of movement. Younger adults have been shown to accelerate rapidly then decelerate to reach a target, whereas older adults accelerated more slowly, reaching peak velocity later in the movement (Seidler-Dobrin et al., 1998). In the current study, average speed of movement was determined by dividing the total movement time by the distance that the cursor travelled to reach each target and therefore it is not possible to determine whether the same pattern of speed changes was evident here.

There were improvements in the PI which indicate a shift in the speed-accuracy trade-off with practice (Figure 4.9, page 95), regardless of group. This indicates that the difference between groups for initial movement speed did not translate into between-group differences in PI AUC. Improvements in PI were not affected by tDCS to M1 in younger or older adults, unlike findings with stroke survivors using a "circuit learning" task (Lefebvre et al., 2012b). In the current study there were no significant differences in PI between the last block of the repeated sequence and the subsequent random block, suggesting that the improvements were a general learning effect. If this finding is confirmed with stroke survivors then it could have implications for rehabilitation as practice on a learning task could induce a shift in the speed-accuracy trade-off which could translate into other, untrained, tasks.

4.5.6 Summary

Clear performance impairments were evident for a number of learning measures for older adults which were not rectified by delivering 20 minutes of 1 mA tDCS to M1. Older adults showed no changes with tDCS for the neurophysiological assessments used, whereas younger adults demonstrated a significant increase in TCI from right to left M1 with bihemispheric tDCS. However, there was no clear relationship between changes in TCI and motor sequence learning performance and the variability in response between participants was high, leading to an overall null effect of tDCS on motor sequence learning. Whether retention of learning on this task is affected by tDCS still requires investigation and will be presented in Chapter 6.

Chapter 5 The effect of electrode arrangement on motor sequence learning and upper limb function in chronic stroke

5.1 Abstract

Background: tDCS has the potential to improve motor control after stroke. However, there are inconsistent results as to the effect of electrode arrangement and it is unclear which is most effective for patients with mild and moderate impairment.

Aims: To systematically assess the effect of tDCS electrode arrangement on motor sequence learning, upper limb function and transcallosal inhibition in chronic stroke survivors.

Methods: A cohort of 24 stroke survivors (3 – 124 months post-stroke, 34 – 76 years of age) completed four sessions of a motor sequence learning task involving a repeated sequence of movements with the paretic arm. In each session tDCS was delivered in a different arrangement (crossover design); i) anodal to ipsilesional M1, ii) cathodal to contralesional M1, iii) bihemispheric, and iv) sham. Upper limb function was assessed pre- and post-stimulation using the JTT and change in TCI (iSP duration) was assessed using TMS.

Results: Participants demonstrated sequence specific learning. Active tDCS did not improve learning, regardless of electrode arrangement. There was a significant improvement in JTT (*vs* sham) after unilateral (anodal or cathodal) tDCS, but not after bihemspheric. There was no effect on TCI and no relationships between TCI and changes in learning or JTT.

Conclusions: Unilateral tDCS is effective for improving upper limb function but not motor sequence learning. Improvements do not appear to be driven by changes in TCI. These findings could have implications for the use of tDCS alongside rehabilitation and guide the design of future studies.

5.2 Introduction

Stroke is a leading cause of adult disability and after current rehabilitation protocols many people are left with impairment and dependent on others for activities of daily living (Dobkin, 2005; Veerbeek et al., 2011). Strategies such as tDCS, with the potential to improve recovery of movement, require investigation.

After unilateral stroke there is commonly an intercortical imbalance in motor activity, with relative under-activity of the ipsilesional M1 and over-activity of contralesional M1 (Murase et al., 2004; Nowak et al., 2009; Takeuchi et al., 2010; Takeuchi and Izumi, 2012; Wessel et al., 2015). The exact purpose of increases in contralesional M1 activity remains unclear (Buetefisch, 2015; Hoyer and Celnik, 2011), but stroke survivors with higher function tend to show better balance in cortical activity, predominantly due to increased ipsilesional M1 activity (Cunningham et al., 2015). Based on the interhemispheric imbalance model, bihemispheric tDCS could hypothetically show greater benefit than unilateral stimulation as the anode is applied to increase excitability of the ipsilesional hemisphere and the cathode to decrease excitability of the contralesional hemisphere concurrently. Motor learning has been shown to improve with cathodal tDCS of contralesional M1 in well recovered stroke survivors using a sequence learning paradigm involving dexterous key press movements (Zimerman et al., 2012) and with bihemispheric tDCS in mild to moderate stroke survivors using a circuit learning paradigm (Lefebvre et al., 2012b). However, the impact of electrode arrangement on motor sequence learning and motor function after stroke is currently unclear. Although the interhemispheric imbalance model provides a rationale for targeting the contralesional M1 during cathodal and bihemispheric tDCS, changes in transcallosal inhibition have only been demonstrated in stroke survivors following bihemispheric tDCS in combination with constraint of the unaffected arm (Bolognini et al., 2011).

The aim of this study was therefore to determine whether, in chronic stroke survivors with upper limb impairment, the electrode arrangement for tDCS impacts on:

1. The rate and amount of motor sequence learning (onset time, anticipations, speed and accuracy),
2. Changes in upper limb function (Jebsen Taylor Test (JTT)),
3. Changes in TCI (iSP duration),
4. Associations between changes in motor sequence learning, JTT performance and TCI.

Based on previous studies, the hypotheses were:

1. The rate of motor sequence learning and the improvement in JTT would be greater with active stimulation compared to sham,
2. Bihemispheric tDCS would provide additional enhancement over unilateral (anodal or cathodal) stimulation,
3. Improvements in motor sequence learning and JTT with active tDCS would be associated with an increase in TCI from ipsilesional M1.

5.3 Methods

5.3.1 Participants

Potential participants were identified between March 2014 and May 2016 from King's College Hospital NHS Foundation Trust, Lewisham and Greenwich NHS Trust, Croydon University Hospital, stroke user groups and word of mouth. In total, 80 stroke survivors underwent an initial screening and agreed to be contacted by the researcher. Of these, 25 participants were eligible and consented to take part (Figure 5.1). Participant characteristics are provided in Table 5.1 (page 121). Time since stroke and stroke location were determined from medical records. All appointments were conducted either in a laboratory at King's College London, or in the Stroke Unit at The Princess Royal University Hospital, King's College Hospital NHS Foundation Trust.

Inclusion criteria were: aged > 18 years; first monohemispheric stroke > 3 months duration; unilateral upper limb weakness and physically able to complete the motor sequence learning task with the affected hand. Exclusion criteria were: contraindications to TMS such as epilepsy or seizures, cardiac pacemakers or metal implants in the head; medications known to alter central nervous system excitability; and cognitive dysfunction sufficient to limit the ability to provide informed consent. All participants gave written informed consent and the study was funded by the Stroke Association, approved by the National Research Ethics Service (13/LO/0965) and adopted by the UK National Institute for Health Research (NIHR) clinical research portfolio (UKCRN ID: 16299).

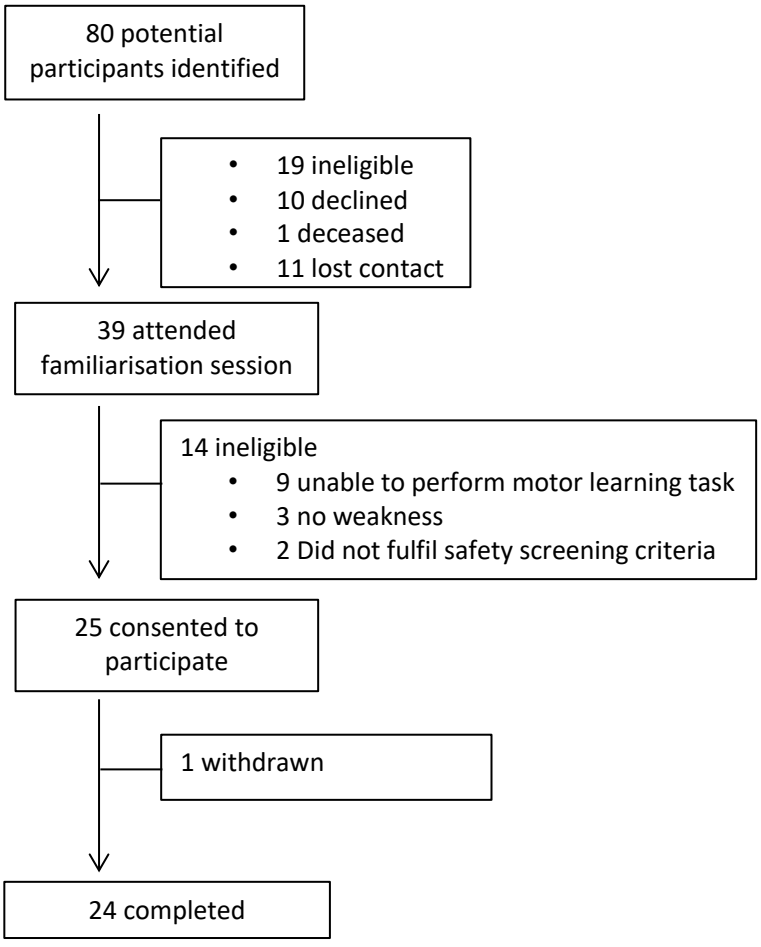


Figure 5.1 Recruitment of participants.

5.3.2 Assessments

Participants attended five sessions in total, with at least one week separating sessions. The time of day was kept as consistent as possible and each session lasted ~1.5 hours.

5.3.2.1 Familiarisation session

Participants practiced the tasks required (motor sequence learning task and JTT), without receiving tDCS, in order to minimise potential differences between sessions due to familiarisation with the protocols. Familiarisation of the JTT involved 10 repetitions of each task, or until performance time stabilised (mean (SD): 7 (2) repetitions). For the motor sequence learning task, participants completed as many repetitions as necessary to ensure that they felt they were comfortable with the use of the computer mouse with the paretic upper limb and understood the purpose of the task (mean (SD): 11 (6) repetitions).

5.3.2.2 Experimental sessions

The remaining four sessions were conducted using a within-subject crossover design with sessions at least one week apart (mean (SD): 11 (7) days) to minimise carry over effects. The crossover design was chosen in an attempt to control for inter-individual variation in upper limb function and ability to learn the movement sequence, and to enable a systematic assessment of differences between electrode arrangements. In each session (Figure 5.2), participants initially performed three repetitions of the JTT, followed by TMS (to localise M1 and assess TCI; see section 5.3.4.2). The tDCS (see section 5.3.4.3) was then delivered whilst participants performed the motor sequence learning task (which took on average 24 minutes to complete). Following completion of the motor sequence learning task the TMS was again delivered to assess TCI and an additional three repetitions of the JTT performed. One participant was unable to tolerate long durations of TMS and so it was used to localise M1 but TCI was not assessed. Two other participants did not undergo TMS (one found it too painful, the other hand a seizure > 30 years

earlier) and M1 was localised using C3/C4 of the 10-20 EEG system. Similarly, this method was used to locate the ipsilesional M1 if it was not possible to elicit MEPs.

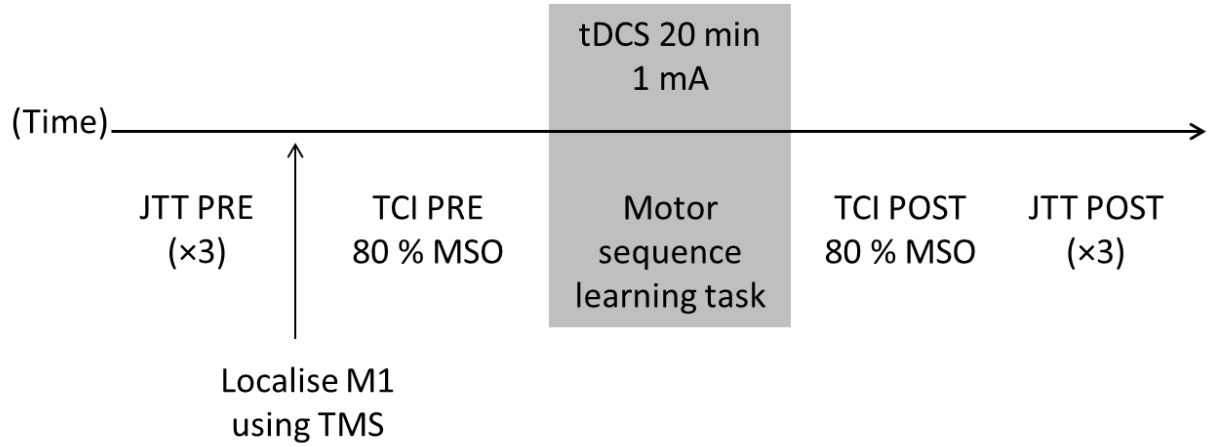


Figure 5.2 Timeline during experimental sessions.
JTT = Jebsen taylor test, TCI = assessment of transcallosal inhibition, MSO = maximum stimulator output, tDCS = delivery of transcranial direct current stimulation.

5.3.3 Motor sequence learning task

This was performed as described in Chapter 3. A sequence of 12 movements was used, consistent with Chapter 4 which tested the effect of tDCS on motor sequence learning in people without stroke. Participants used their affected hand to perform the task in order to determine the effect of tDCS on motor sequence learning with the paretic limb.

In each experimental session, participants initially completed two practice sequences to re-familiarise them with the movement of the mouse to the targets. They were then reminded that they would repeat a sequence of 12 movements, 25 times, and that they could anticipate target appearance if they knew which target would illuminate next. The sequence for each participant and session was chosen randomly from a pool of eight sequences, ensuring that a different one was performed in each session. The pool of sequences was the same as for Chapter 4 (healthy adults). Following completion of the 25 repetitions of the sequence, two random sequences (12

movements) were performed to distinguish between general learning and sequence specific learning effects. Finally, one additional repetition of the trained sequence was completed.

As described in Chapters 3 and 4, the OT was recorded as the time between the target illuminating and the cursor leaving the central square and the PI calculated based on speed and accuracy (PL) of cursor movement. These values were normalised to the first repetition and averaged across consecutive repetitions to form 13 blocks.

Learning was assessed as the change in normalised OT and PI over the blocks, the AUC (which takes into account both rate and amount of change), and the specificity of learning as the difference between the last block of the repeated sequence and the random block. An "anticipation" was recorded when the cursor left the central square prior to target illumination. The total number of accurate anticipations was determined by summing the anticipations over the 25 repetitions to give a single value for each participant.

5.3.4 Stimulation of primary motor cortex

5.3.4.1 Setup

As described in Chapter 4, TMS was used to determine the position of the M1 representation of each FDI muscle for placement of the tDCS electrodes and to assess TCI at baseline and immediately post-stimulation. Muscle activity (EMG) was recorded from each FDI and acquired and processed as specified in Chapter 4.

A figure-of-eight coil (70 mm diameter) with a Magstim 200 (Princess Royal University Hospital) or 200^2 (Guy's Campus) stimulator (Magstim Company, UK) was used to elicit MEPs, while participants rested their hands prone on a pillow on their laps. The optimal position for evoking MEPs in the relaxed FDI was established in each session and marked with a water-soluble marker directly on the scalp to ensure consistent coil placement. The RMT was determined in the first

session to give an indication of the balance in excitability across hemispheres. This was done in a standard manner, as the minimum intensity required to elicit an MEP of ≥ 50 μV in the relaxed FDI from at least 4 out of 8 consecutive stimuli.

5.3.4.2 Transcallosal inhibition

As described in Chapter 4, TCI was assessed using a TMS intensity of 80 % MSO. Participants were instructed to activate the FDI muscle at ~75 % of their maximal effort (isometric contraction) while single pulse stimuli were delivered to the ipsilateral M1. Twenty stimuli were delivered to each M1 before and immediately following performance of the motor sequence learning task. The duration of TCI was calculated as specified in Chapter 4, using Signal 4.07 (CED, UK). An average duration was calculated for each hemisphere at baseline and post-stimulation in each session. The change in iSP duration was calculated as $iSP_{POST} - iSP_{PRE}$. If the participant could not sustain a voluntary contraction of the paretic hand then iSP duration was assessed for the "unaffected" FDI only (representing ipsilesional to contralesional M1 TCI).

To obtain an indication of whether corticospinal excitability changed as a result of tDCS the peak-to-peak MEP amplitude (mV) from the contralateral FDI was recorded during each TCI trial using Signal 4.07 (CED, UK; Appendix E).

5.3.4.3 Transcranial direct current stimulation

For the experimental sessions tDCS was delivered for the first 20 minutes of the motor sequence learning task at 1 mA using a constant current stimulator (Neuroconn, Rogue Resolutions, UK) with two carbon electrodes encased in 5 x 5 cm square saline-soaked sponges (current density 0.04 $mA.cm^{-2}$). For anodal tDCS the anode was placed over the ipsilesional M1 (FDI "hotspot") and the cathode over the contralateral supraorbital ridge, for cathodal tDCS the cathode was placed over the contralesional M1 and the anode over the contralateral supraorbital ridge, and for bihemispheric tDCS the anode was placed over ipsilesional M1 and

the cathode over contralesional M1 (Figure 5.3). This study was designed as a single-blind, sham controlled crossover trial. For sham tDCS the current was ramped up, delivered for 30 s in either of the electrode arrangements (randomly chosen), then turned off. The order of tDCS conditions was randomised across participants using a Latin square design. In order to blind participants to the tDCS electrode arrangement, sponges were placed on all four scalp locations (bilateral M1, bilateral supraorbital ridge), but only two of the sponges contained electrodes.

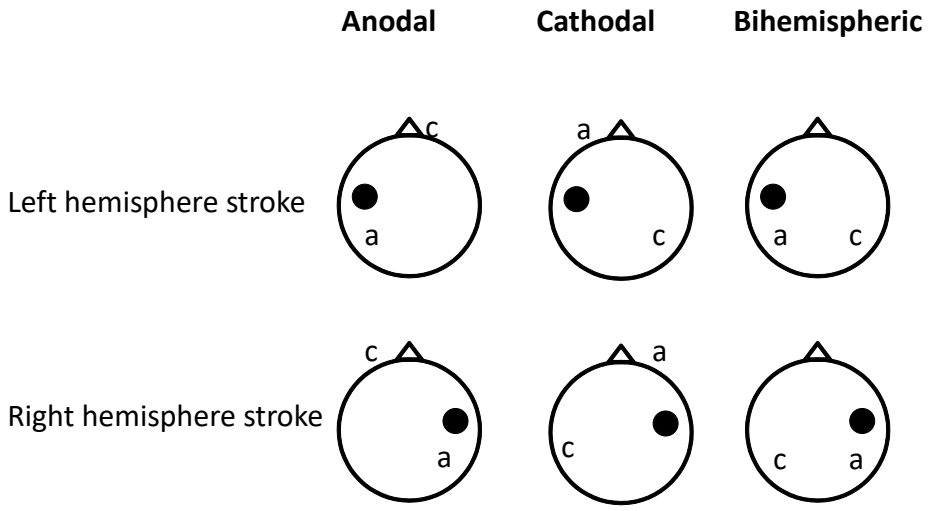


Figure 5.3 Representation of tDCS electrode configurations.
Black sphere indicates hemisphere of stroke, a = anode, c = cathode.

5.3.5 Paretic upper limb function

The JTT (Jebsen et al., 1969) was used as a marker of paretic upper limb function before and after tDCS and performance of the motor sequence learning task. This timed test assesses gross motor function (e.g. moving cans) and dexterity (e.g. picking up paperclips). The writing subsection was removed as is standard practice for this population.

The time (s) was averaged across the three repetitions and the percentage change in time for post-stimulation compared with pre-stimulation was calculated for each session (($Time_{POST}$ – $Time_{PRE}$) / $Time_{PRE}$ × 100). Additionally, the percentage change in time for the "fine motor" and

"gross motor" subsections of the JTT were calculated separately to determine whether the dexterity requirements of the tasks influenced the response to tDCS (Hummel et al., 2010).

5.3.6 Statistical Analysis

Based on a previous motor sequence learning study (Zimerman et al., 2012) it was estimated that for an effect size of 0.67 at least 20 participants would be required to find a difference in learning (OT AUC) between active and sham stimulation with $\alpha = 0.05$ and power of 80 %.

Analysis was conducted using SPSS 21.0 (IBM Inc.). Normality of the residuals was assessed using Kolmogorov-Smirnov tests and visual inspection of frequency histograms and non-parametric tests utilised if the assumption of normality was not sustained and transformation was ineffective. Violations of sphericity were corrected using the Greenhouse-Geisser correction. Data are presented as mean ± SEM and significance was set at $p < 0.05$, unless otherwise specified.

5.3.6.1 Motor sequence learning task

A 12 BLOCK × 4 TDCS rmANOVA was used to determine whether normalised OT or PI changed with training and whether this was dependent on tDCS electrode arrangement (sham, anodal, cathodal, bihemispheric). A 2 BLOCK × 4 TDCS rmANOVA was used to determine whether normalised OT or PI differed between the last block of the repeated sequence and the random block (sequence specific learning).

To compare OT AUC, PI AUC and the number of anticipations directly between active stimulation conditions the values for anodal, cathodal and bihemispheric were expressed relative to the sham stimulation condition, either as a percentage (% sham) or by subtracting the sham value (-sham). A 3 TDCS rmANOVA (or Friedman test) was conducted with these relative values. One

sample t-tests (or Wilcoxon signed rank tests) were used to determine whether values for active stimulation differed from sham (100 % or 1).

5.3.6.2 Upper limb function

A 4 TDCS rmANOVA was used to determine whether there was an effect of tDCS condition (sham, anodal, cathodal, bihemispheric) on percentage change in JTT time (% Δ JTT). To determine whether any differences in response between active electrode arrangements depended on the nature of the task (i.e. “fine motor” vs “gross motor”) a 3 TDCS × 2 DEXTERITY rmANOVA was used with change expressed relative to sham by subtraction (- sham).

5.3.6.3 Transcallosal inhibition

A 4 TDCS rmANOVA was used for the change in iSP duration from each hand separately, to determine whether the change in TCI was dependent on tDCS condition.

5.3.6.4 Relationships between variables

Pearson correlations were used to assess for relationships between the change in iSP duration (ipsilesional to contralesional M1 TCI) and learning (OT AUC and OT difference between last repeated block and random block) or JTT change expressed relative to sham. Due to multiple correlations an adjusted significance of $p < 0.01$ was used.

5.4 Results

Participant characteristics are presented in Table 5.1. One participant withdrew from the study before completion (#12 due to a headache after first stimulation session (sham tDCS)), leaving 24 for analysis. Participants commonly reported a transient itching sensation during tDCS or no sensation. There were no other reported adverse effects from TMS or tDCS.

Table 5.1 Participant characteristics.

Participant	Sex	Age (years)	Time since stroke (months)	Affected hand	Dominant hand	Initial JTT (s)	Type of stroke	Location of stroke	MEP status (+/-)
1	M	52	46	R	R	77.5	H	C	+
2	M	67	124	R	R	46.0	I	S	+
3	M	62	32	R	R	44.7	I	S	+
4	F	57	43	R	R	45.3	I	S	-
5	M	76	10	L	L	29.7	I	C	-
6	M	39	13	L	R	94.1	I	S	+
7	M	65	3	L	L	65.6	I	C	+
8	M	39	54	L	R	131.2	H	S	+
9	F	59	6	L	R	52.25	I	C/S	-
10	M	66	52	R	R	281.3	I	C	
11	F	34	26	R	R	314.11	I	S	-
12[a]	M	81	4	R	R	43.07	I	S	+
13	M	63	6	L	R	44.16	I	S	+
14	M	63	5	L	R	33.09	H	S	+
15	F	61	9	R	R	36.19	I	C	+
16	M	62	7	L	R	30.06	I	C	+

Participant	Sex	Age (years)	Time since stroke (months)	Affected hand	Dominant hand	Initial JTT (s)	Type of stroke	Location of stroke	MEP status (+/-)
17	M	36	3	R	R	61.94	H	S	-
18	M	67	4	R	R	99.06	I	C	+
19	M	56	7	L	R	54.01	I	S	+
20	M	69	3	R	R	40.26	I	S	+
21	M	74	3	L	R	43.95	I	S	+
22	M	50	7	L	R	132.27	I	S	-
23	F	76	20	R	R	52.38	I	S	-
24	M	47	3	R	R	34.67	I	S	+
25	M	74	3	L	R	47.59	I	S	+
Min		34	3			29.7			
Max		81	124			314.1			
Mean (SD)		59.8 (13.1)	19.7 (27.4)			77.4 (72.2)			
Median		62	7			47.6			
Count	20 M/ 5 F			13 R/ 12 L	23 R / 2 L		4 H / 21 I	8 C / 17 S	17+ / 7-

[a] withdrawn from study prior to completion. JTT=Jebsen Taylor test time, M = male, F = female, R = right, L = left, I = Ischaemic, H = Haemorrhagic, S = subcortical, C = cortical, SD = standard deviation. MEP status refers to presence (+) or absence (-) of MEPs in affected FDI in response to TMS (N.B data not available for patient #10 as no TMS delivered).

5.4.1 Corticospinal excitability

Resting motor threshold was significantly higher for the ipsilesional M1 (median (range) 63.5 (32 – 100) % MSO) than the contralesional (52.5 (31 - 80) % MSO, Wilcoxon signed rank test p = 0.002). Similarly, baseline MEPs recorded during TCI assessment (at 80 % MSO) were significantly smaller from the ipsilesional M1 (median (range) 0.94 (0.14 – 6.35) mV) than the contralesional (3.42 (0.72 – 6.26) mV, Wilcoxon signed rank test, p = 0.013). This indicates an overall imbalance in corticospinal excitability across the hemispheres, as expected.

5.4.2 Motor sequence learning task

5.4.2.1 OT over the blocks

The absolute OT of the first repetition did not differ across the sessions (Friedman test, p = 0.950; mean OT (SD) = 0.49 (0.12) s). The 4 TDCS by 12 BLOCK rmANOVA revealed an effect of BLOCK ($F_{2.3,51.7}$ = 14.956, p < 0.001), but no effect of TDCS ($F_{3,69}$ = 0.839, p = 0.477) and no interaction ($F_{10.6,244.0}$ = 0.932, p = 0.508). This indicates that OT reduced over the blocks of training on the repeated sequence, irrespective of the tDCS condition (Figure 5.4).

5.4.2.2 Specificity of sequence learning

There was a significant increase in OT between the last block of the repeated sequence and the random block (effect of BLOCK: $F_{1,23}$ = 45.117, p < 0.001) indicating that improvements in OT were specific to the trained sequence (Figure 5.4). There was no effect of TDCS ($F_{3,69}$ = 0.539, p = 0.657) or interaction between TDCS and BLOCK ($F_{3,69}$ = 0.753, p = 0.524) indicating that the specificity of sequence learning was not affected by the tDCS condition.

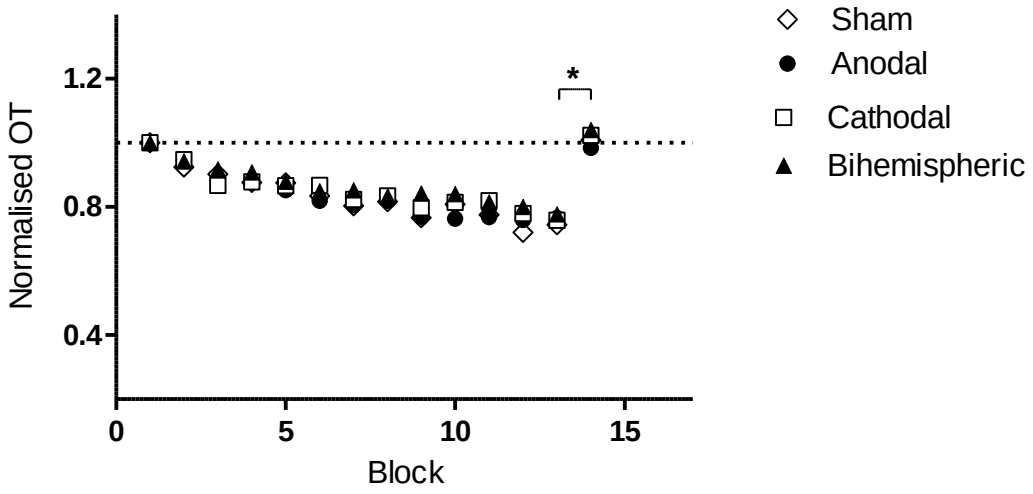


Figure 5.4 Change in mean normalised OT with training of the movement sequence.
Block 14 represents the random block. There was a significant effect of block ($p < 0.001$) as OT reduced with training. * significant difference between the last block of the repeated sequence and the random block ($p < 0.001$) across all conditions.

5.4.2.3 OT AUC

When OT AUC was expressed relative to sham stimulation (% sham), the 3 TDCS rmANOVA showed no effect of TDCS ($F_{2,46} = 1.094$, $p = 0.344$;Figure 5.5). When pooled across the three active tDCS conditions (102.7 ± 2.4 %) the one-sample t-test showed no difference from sham (100 %; $t(23) = 1.129$, $p = 0.271$), indicating that the OT AUC did not improve with active tDCS.

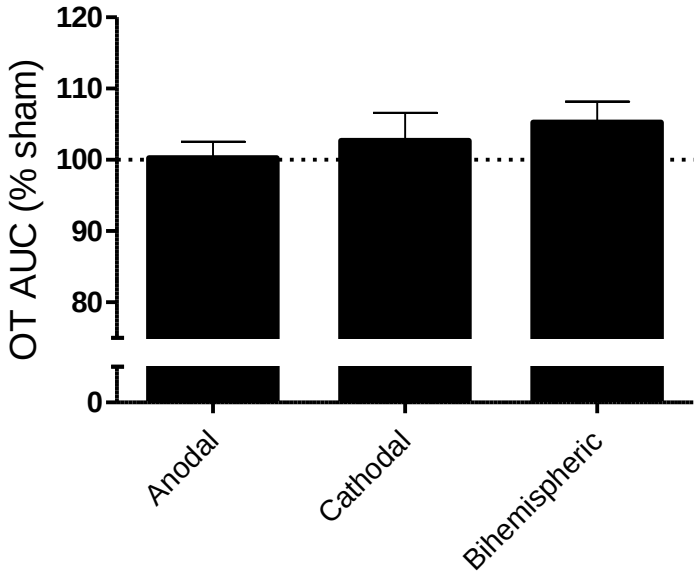


Figure 5.5 OT AUC expressed relative to sham stimulation (mean ± SEM).
Values < 100 % indicate improved learning. OT AUC was not affected by tDCS condition.

5.4.2.4 Total anticipations

There was a wide range across participants for the number of anticipations of target appearance, with some showing none and the greatest total being 155. Therefore, the total anticipations for each active condition for each participant were expressed relative to sham stimulation by subtracting the total anticipations during the sham stimulation session (- sham). Values > 0 indicate more anticipations of target appearance than the sham condition.

The 3 TDCS rmANOVA showed no effect of TDCS ($F_{2,46}$ = 1.281, p = 0.287; Figure 5.6). When data were pooled across active tDCS conditions (median: 0.3, range -40 - 17) they were not normally distributed. A one-sample Wilcoxon signed rank test found no difference from sham (0; p = 0.276), indicating that active tDCS did not affect the number of anticipations.

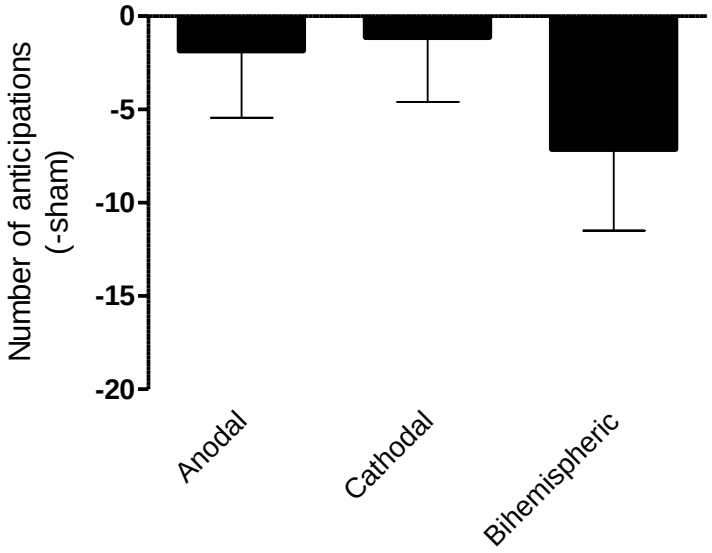


Figure 5.6 Number of anticipations (-sham) for each active tDCS condition (mean ± SEM). Negative values indicate less anticipations than sham session. There was no effect of tDCS condition (p 0.287) and no difference from sham when pooled across active tDCS conditions (p = 0.276).

5.4.2.5 Speed-accuracy trade-off (PI)

The 4 TDCS × 12 BLOCK rmANOVA with log-transformed data showed no effect of BLOCK ($F_{5.6,129.5} = 1.456$, $p = 0.202$) or TDCS ($F_{3,69} = 0.202$, $p = 0.894$) and no interaction ($F_{11.9,273.8} = 1.370$, $p = 0.181$). There was also no difference between the last block of the repeated sequence and the random block (effect of BLOCK: $F_{1,23} = 0.351$, $p = 0.560$) and no interaction with TDCS ($F_{3,69} = 0.249$, $p = 0.862$). This indicates that there was no change in the speed-accuracy trade-off with training or with tDCS. Figure 5.7 shows non-transformed data for each tDCS condition.

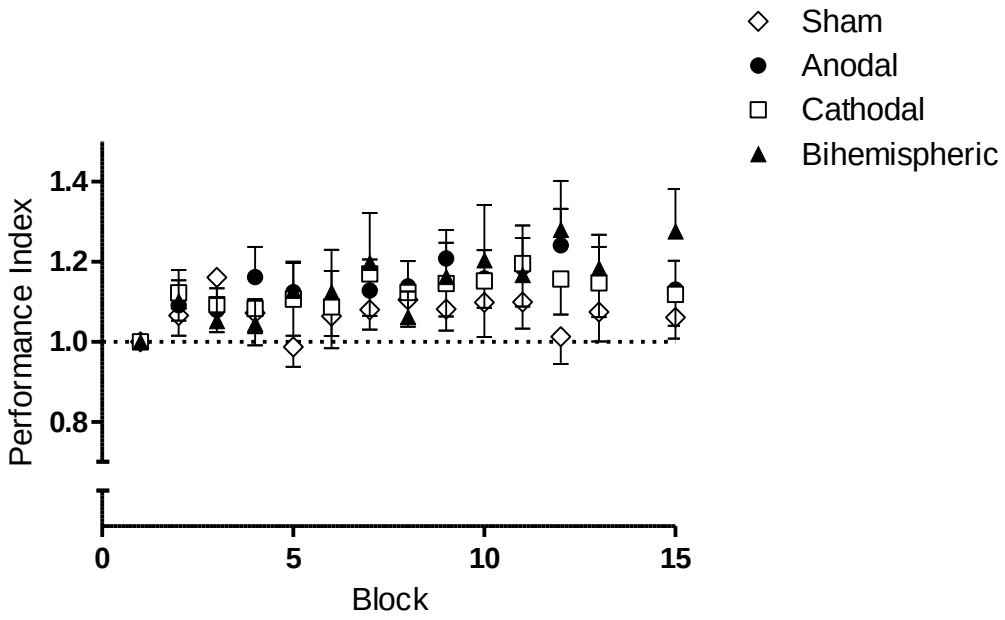


Figure 5.7 PI (non-transformed) over the blocks (mean ± SEM).
There was no change in PI over the blocks for any tDCS condition ($p > 0.05$). Block 15 represents random block. There was no difference in PI between the last repeated and the random block ($p = 0.560$).

The PI AUC expressed relative to sham (% sham) was not normally distributed and transformation was ineffective. The 3 TDCS Friedman test showed no effect of TDCS ($p = 0.959$; Figure 5.8) and when data were pooled across active tDCS conditions (median 101.8 %) a one-sample Wilcoxon signed rank test found no difference from sham (100 %; $p = 0.493$), indicating that active tDCS did not induce changes in the AUC for the speed-accuracy trade-off.

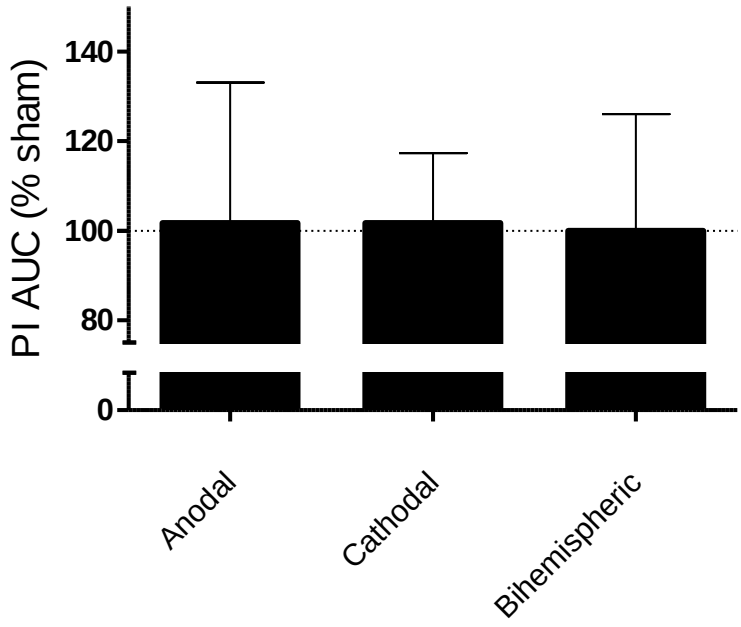


Figure 5.8 PI AUC (% sham) for each active tDCS condition.
Data are shown as median (interquartile range). Values > 100 % indicated improved speed-accuracy trade-off over sham. There was no effect of tDCS condition ($p = 0.959$).

5.4.3 Upper limb function

Initial JTT time varied considerably across participants (see Table 5.1) indicating a range in upper limb function. There was no effect of SESSION on the baseline (pre-stimulation) JTT indicating consistency across the four sessions (Friedman test p = 0.246).

The 4 TDCS rmANOVA showed an effect of TDCS on the % Δ JTT time ($F_{3,69}$ = 5.194, p = 0.003; Figure 5.9). *Post-hoc* comparisons (one-tailed paired samples t-tests, with Bonferroni correction) showed that JTT time was significantly reduced after anodal (-7.7 ± 2.0 %, p = 0.006, effect size d = 1.0) and cathodal (-8.2 ± 2.5 %, p = 0.003, d = 0.7) tDCS compared with sham (0.7 ± 1.4 %), but not after bihemispheric (-2.2 ± 1.9 %, p = 0.371, d = 0.4).

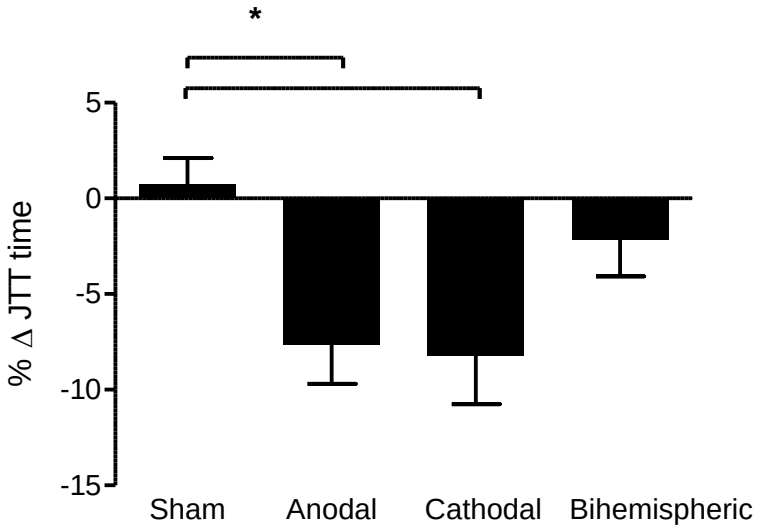


Figure 5.9 Change in JTT time for each tDCS condition (mean ± SEM).
There was an effect of tDCS (p = 0.003), * significant improvement compared with sham (p < 0.05 with Bonferroni correction).

When divided into "fine motor" and "gross motor" subsections, expressed relative to sham by subtraction (- sham), there was a tendency toward an effect of TDCS ($F_{2,46}$ = 3.108, p = 0.054) as there tended to be a greater improvement with anodal or cathodal tDCS compared with bihemispheric. There was no difference between "fine motor" and "gross motor" subsections (effect of DEXTERITY; $F_{1,23}$ = 2.090, p = 0.162) or interaction between TDCS and DEXTERITY

($F_{1.6,37.1}$ = 0.017, p = 0.967) indicating that the improvements with unilateral tDCS were across all task types (Figure 5.10).

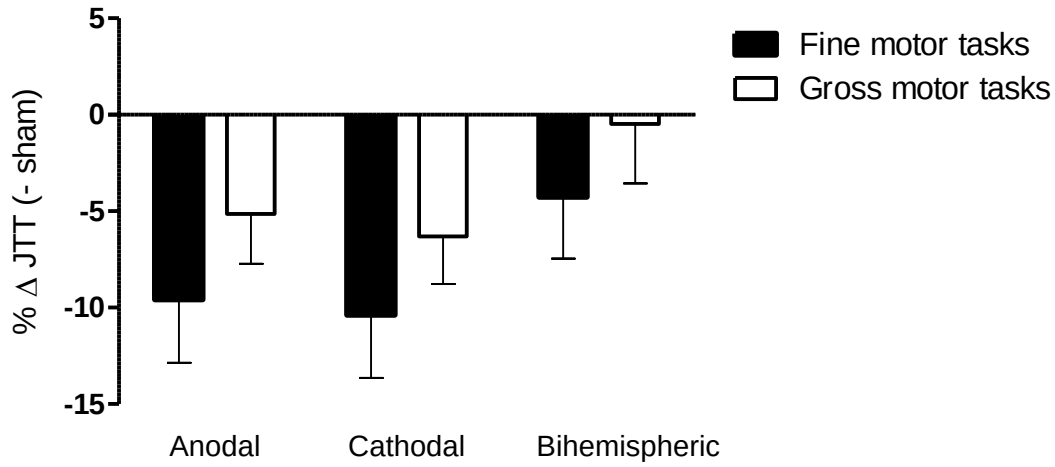


Figure 5.10 JTT change for fine and gross motor task subsections (mean ± SEM).
JTT change was expressed relative to sham by subtraction (-sham). There was a tendency for greater improvement with anodal or cathodal tDCS compared with bihemispheric (p = 0.054) but no difference between subsections (p = 0.162).

5.4.3.1 JTT subgroup analyses

To further assess the effect of tDCS on upper limb function, subgroup analyses were conducted to examine differences based on time since stroke (< 6 months post-stroke *vs* > 6 months post-stroke), stroke location (subcortical *vs* cortical) and hand affected (dominant *vs* non-dominant), using JTT change expressed relative to sham by subtraction (- sham), with age and initial JTT entered as potential co-variates.

There was no effect of TIME SINCE STROKE (< 6 months n = 10, > 6 months n = 14; $F_{1,20}$ = 1.211, p = 0.284), and no interaction between TDCS and TIME SINCE STROKE ($F_{2,40}$ = 1.743, p = 0.188). This suggests that the within-session improvements in JTT with tDCS were not dependent on whether the stroke was recent (< 6 months prior) or not (Figure 5.11).

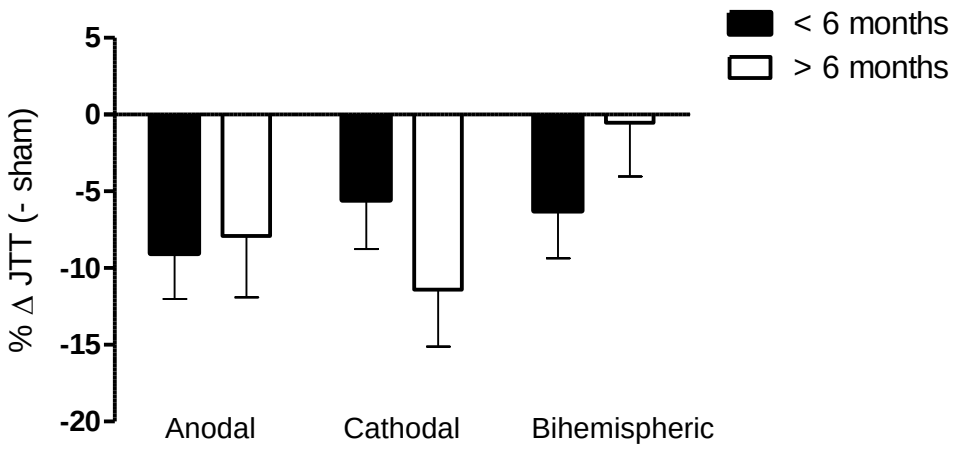


Figure 5.11 JTT change grouped by time since stroke (mean ± SEM).
JTT change was expressed relative to sham by subtraction (-sham). Time since stroke (< or > 6 months) did not affect improvements with tDCS (p = 0.284).

There was a significant effect of HAND ($F_{1,20}$ = 6.527, p = 0.019), but no interaction with TDCS ($F_{2,40}$ = 0.656, p = 0.524). This suggests that the group with their previously dominant hand affected (n = 14) had a greater improvement across all active conditions than the group with the non-dominant hand affected (n = 10; Figure 5.12).

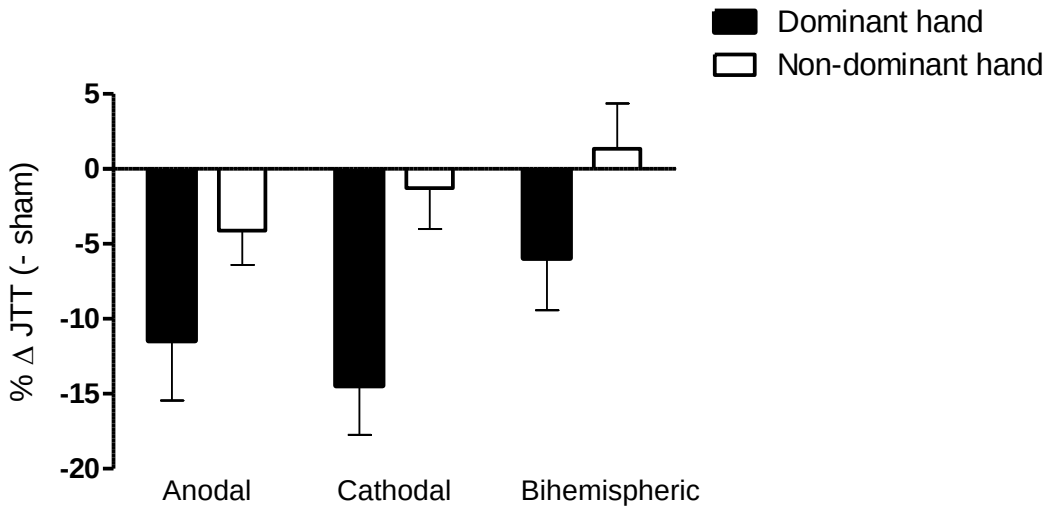


Figure 5.12 JTT change grouped by hand affected (mean ± SEM).
JTT change was expressed relative to sham by subtraction (-sham). The group with the dominant hand affected showed greater improvements across all active tDCS conditions (p = 0.019).

There was a significant effect of LOCATION ($F_{1,20}$ = 16.032 , p = 0.001), but no interaction with TDCS ($F_{2,40}$ = 0.611 , p = 0.548). This suggests that the group with stroke affecting the cortical structures of the brain (n = 8) demonstrated greater improvement across all active conditions than the group with only subcortical structures affected (n = 16; Figure 5.13).

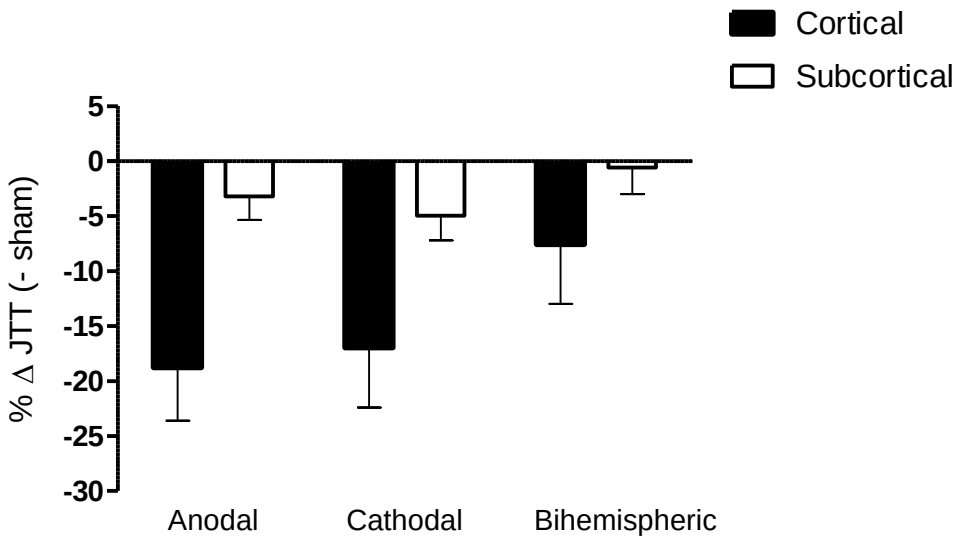


Figure 5.13 JTT change grouped by stroke location (mean ± SEM).
JTT was expressed relative to sham by subtraction (-sham). The group with cortical structures affected showed greater improvements across all active tDCS conditions (p = 0.001).

5.4.4 Transcallosal inhibiton

The change in TCI was assessed from the ipsilesional to contralesional M1 ("unaffected" FDI) for 21 participants, and from the contralesional to ipsilesional M1 (affected FDI) for 11 participants as the remainder were unable to produce consistent EMG activity with the paretic hand. A representative EMG trace is shown in Figure 5.14.

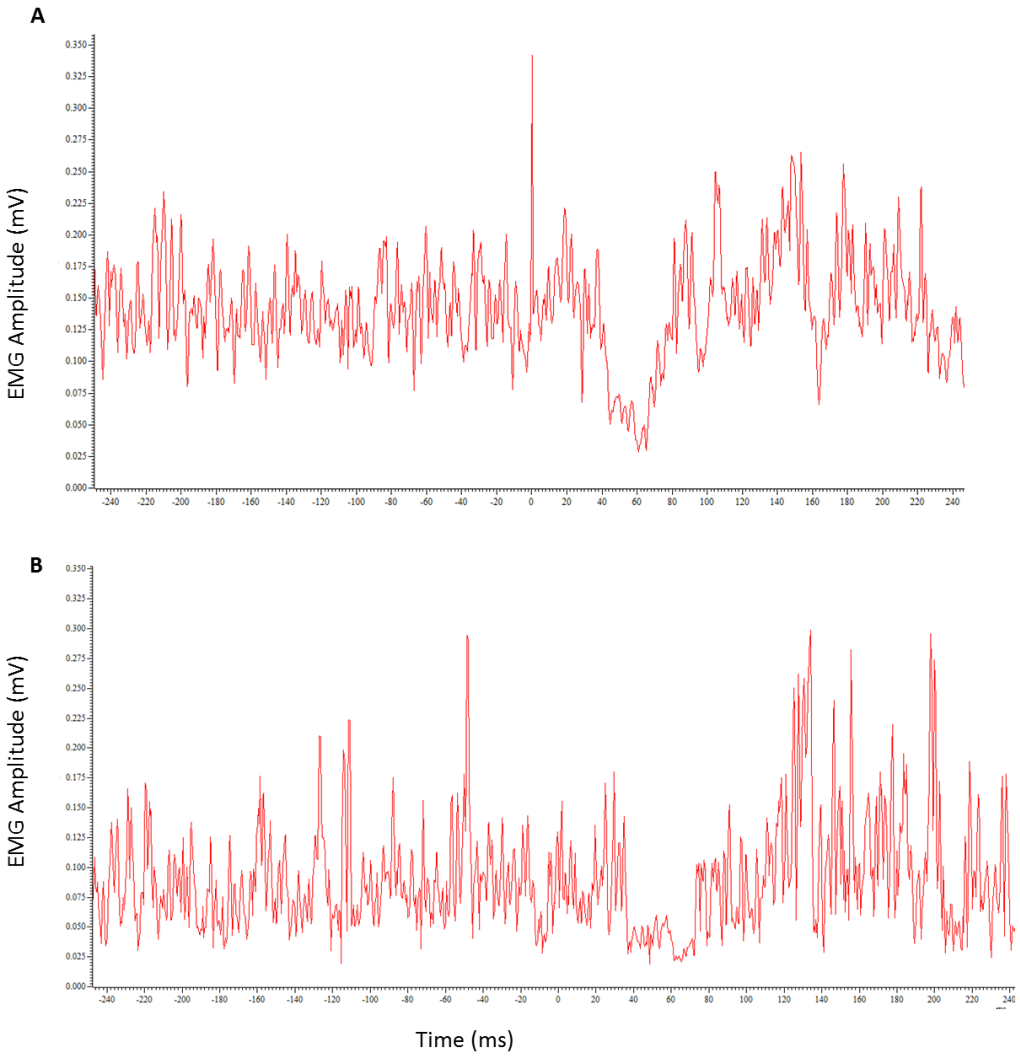


Figure 5.14 Representative EMG trace showing average waveform with silent period.
A. EMG from "unaffected" FDI representing ipsilesional to contralesional M1 TCI. **B.** EMG from "affected" FDI representing contralesional to ipsilesional M1 TCI.

To ensure that voluntary activation (EMG) was consistent pre-post stimulation and across sessions a 4 TDCS × 2 TIME rmANOVA was used for the RMS EMG activity in the 450 ms prior to the stimulus for each hand separately. For the unaffected hand (ipsilesional to contralesional M1 TCI) there was no effect of TDCS ($F_{3,60}$ = 1.838, p = 0.150) or TIME ($F_{1,20}$ = 1.029, p = 0.323) and no interaction ($F_{3,60}$ = 0.290, p = 0.832). Similarly, for the affected hand (contralesional to ipsilesional M1 TCI) there was no effect of TDCS ($F_{1.8,17.6}$ = 0.105, p = 0.877) or TIME ($F_{1,10}$ = 0.166, p = 0.692) and no interaction ($F_{1.7,17.2}$ = 0.885,p = 0.416). These results show that muscle activity was consistent.

The 4 TDCS rmANOVA showed no effect of TDCS on the change in iSP duration from either hand (ipsilesional to contralesional M1 TCI, n = 21: $F_{3,60}$ = 1.157, p = 0.334; contralesional to ipsilesional M1 TCI, n = 11: $F_{3,30}$ = 0.352, p = 0.788), indicating that TCI was not significantly altered as a result of tDCS (Figure 5.15 and Appendix C).

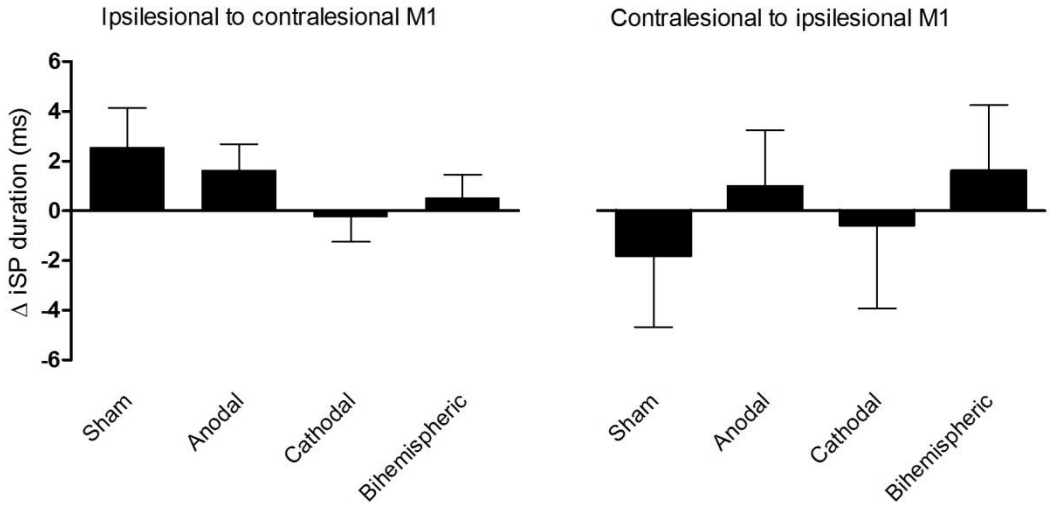


Figure 5.15 Change in TCI from each hemisphere (iSP duration, mean ± SEM) for each tDCS condition. There was no effect of tDCS for either hemisphere (p > 0.3).

5.4.5 Relationships between variables

Full results from the Pearson correlations are shown in Table D2, Appendix D. There were no significant correlations between the change in iSP duration (ipsilesional to contralesional M1 TCI) and OT AUC or OT difference between last repeated and random block, expressed relative to sham, for any active tDCS condition (p > 0.07; Figure 5.16). Similarly, there were no significant correlations between the change in iSP duration and the change in JTT, expressed relative to sham (p > 0.3; Figure 5.17).

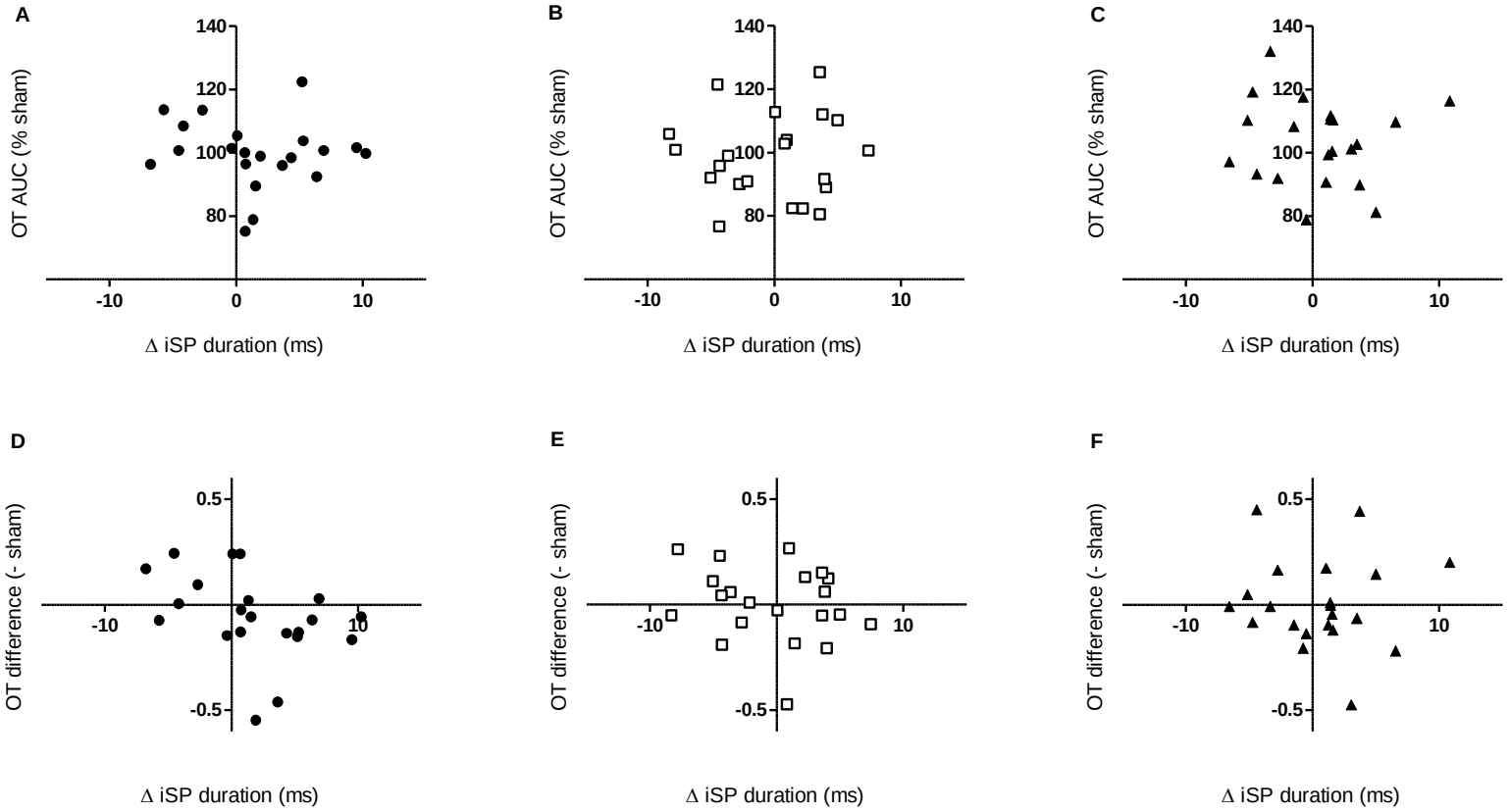


Figure 5.16 Relationship between change in TCI from ipsilesional to contralesional M1 and learning.
Top row: OT AUC (% sham), Bottom row: OT difference between last block of repeated sequence and random sequence, expressed relative to sham session by subtraction (-sham)
A,D. Anodal (filled circles), **B,E.** Cathodal (open squares), **C,F.** Bihemispheric (filled triangles) tDCS. There were no significant correlations.

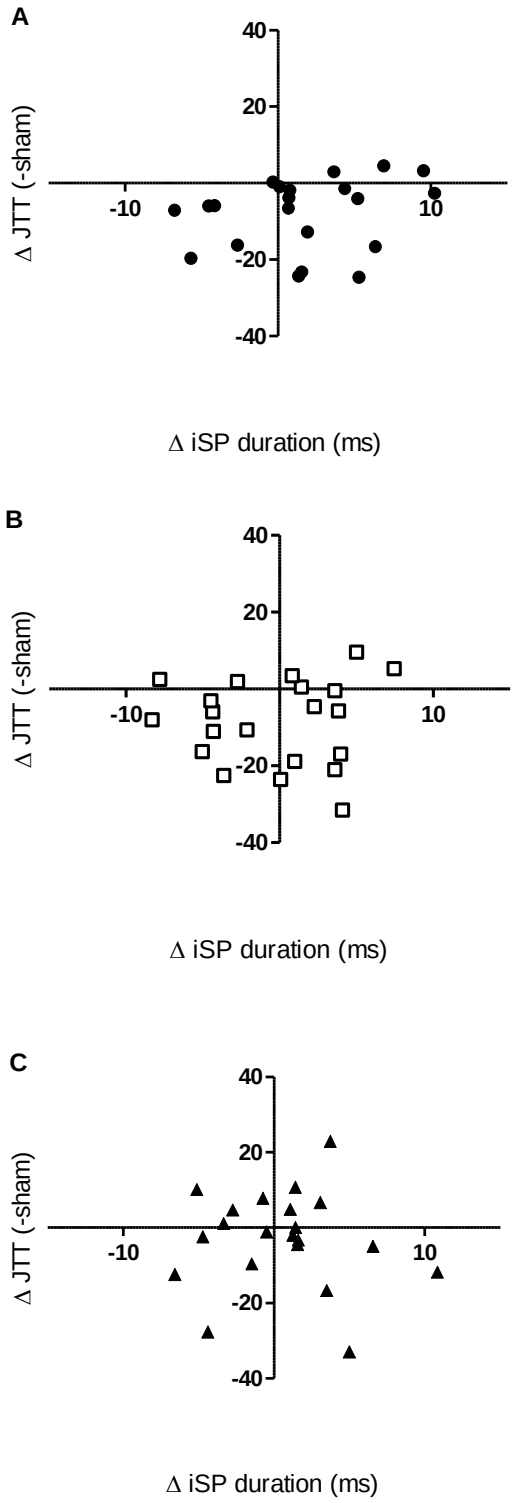


Figure 5.17 JTT change (-sham) as a function of change in TCI from ipsilesional to contralesional M1. **A.** Anodal (filled circles), **B.** Cathodal (open squares), **C.** Bihemispheric (filled triangles) tDCS. There were no significant correlations.

Since JTT was found to improve following unilateral tDCS, but not after bihemispheric, Pearson correlations were also used to assess whether the response to each active stimulation condition correlated with the response to either of the other active conditions. The improvement with anodal tDCS correlated with cathodal tDCS ($R = 0.61$, $p = 0.002$), but neither unilateral condition correlated with bihemispheric (anodal with bihemispheric $R = 0.37$, $p = 0.075$, cathodal with bihemispheric $R = 0.13$, $p = 0.542$). See Figure 5.18.

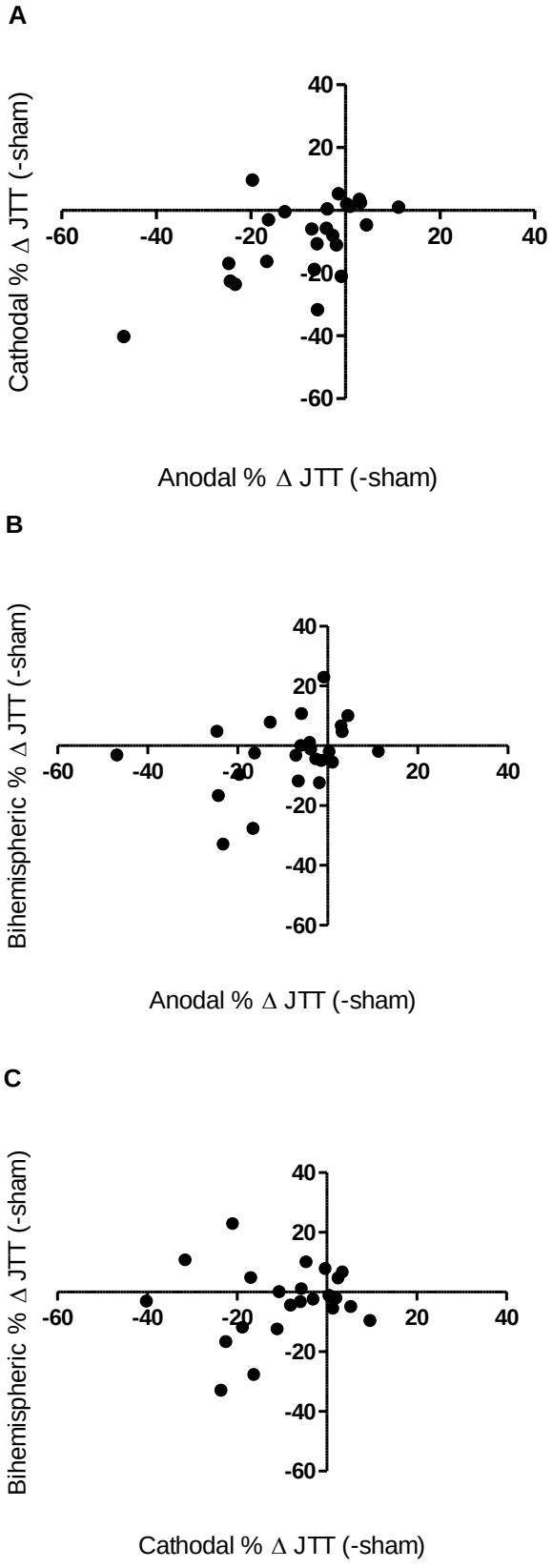


Figure 5.18 The relationship between tDCS electrode arrangements for JTT change (-sham). **A.** anodal compared with cathodal, **B.** anodal compared with bihemispheric, **C.** cathodal compared with bihemispheric. There was a significant correlation between anodal and cathodal only (R = 0.61, p = 0.002).

5.5 Discussion

The main finding of this study was that unilateral (anodal or cathodal) tDCS improved paretic upper limb JTT performance within a session but that bihemispheric stimulation did not. This is the first study to demonstrate a clear effect of electrode arrangement on functional tasks in chronic stroke survivors, using a within-subject design. However, there was no effect of tDCS on motor sequence learning or TCI. Similarly, there was no relationship between change in TCI and change in JTT, suggesting that increases in inhibition from the ipsilesional to contralesional M1 do not underlie the response to tDCS of M1 in this population.

5.5.1 No effect of tDCS on motor sequence learning

Although there have been numerous studies of the effect of tDCS on motor function, there is limited research regarding the effect on motor sequence learning with the paretic arm. This is likely due to a lack of learning paradigms that can be performed with a paretic arm. The current study utilised a novel paradigm requiring gross arm movements that could be performed by patients with moderate upper limb impairment. Significant improvements in movement preparation, i.e. reduction in OT, occurred with learning of the movement sequence. However, tDCS of M1 was not found to significantly alter sequence learning, regardless of the electrode configuration. Similarly, the speed-accuracy trade-off (PI) was unaffected by tDCS.

Two previous studies have found improvements in learning with active tDCS in comparison with sham. Zimerman et al. (2012) demonstrated an increase in the number of correct sequences performed with the affected hand during and following cathodal tDCS of contralesional M1 for a sample of 12 participants with mild impairment. Lefebvre et al. (2012b) found improvements in the PI for a circuit task with bihemispheric tDCS for a sample of 18 participants with mild and moderate impairment. It is unclear why the findings of the current

study differ in comparison with these previous studies. It is possible that the paradigm utilised here may not have been sensitive enough to detect improvements with tDCS. The sequential tap task used by Zimerman et al. required fine finger control, and the circuit task used by Lefebvre et al. likely had a higher accuracy requirement than the current task. Lefebvre et al. used a longer stimulation period (30 minutes) and larger electrodes (35 cm^2) which could also account for the stronger effects, but Zimerman et al. used the same stimulation parameters as the current study. Alternatively, it may be that the motor cortex was not the optimal location to stimulate for the learning strategy used by the participants in this study for this task. However, neither Zimerman et al., nor Lefebvre et al., directly compared results with the other common electrode arrangements. The lack of overall effect of tDCS on learning in the current study means that it is not possible to conclude whether one electrode arrangement provides greater benefit to motor sequence learning with the paretic arm than another.

Surprisingly there was no change in the speed-accuracy trade-off (PI) with training of the movement sequence (Figure 5.7, page 126). This suggests that although participants were able to anticipate target appearance and prepare their movements in advance, the quality of their movement did not change with practice. This may be because, in this explicit learning task, participants were more focused on the time to leave the central square and anticipation of target appearance than they were on the speed and accuracy of their movements once the cursor left the central square. It remains to be seen whether this would be different if learning was implicit instead of explicit. Alternatively, improvements with training may have been masked by fatigue as participants used their paretic arm. However, if this was the case then it is surprising that tDCS was ineffective at reducing this "fatigue effect".

5.5.2 Improvement in upper limb function (JTT) following unilateral tDCS

A recent meta-analysis (Kang et al., 2016) demonstrated overall significant effects for each of the electrode arrangements with regard to improved motor skill and performance after stroke.

The magnitude of the effect size was similar across conditions (anodal = 0.59, cathodal = 0.60, bihemispheric = 0.68). This would appear to suggest that the electrode arrangement has little influence on the response to tDCS. However, the current study utilised a within-subject design to directly compare across electrode arrangements in the same individuals and did find differences, with JTT improving following anodal tDCS of ipsilesional M1 or cathodal tDCS of contralesional M1, but not after bihemispheric tDCS (Figure 5.9, page 128). Anodal tDCS had an effect size of 1.0 (Cohens d), cathodal was 0.7 and bihemispheric was 0.4. Previous studies have also found improvements in JTT performance with active tDCS (Fregni et al., 2005; Hummel et al., 2005; Mahmoudi et al., 2011), but those which have attempted to systematically compare JTT improvements across electrode arrangements have been limited by small sample sizes (Fregni et al., 2005; Mahmoudi et al., 2011) making it difficult for them to draw conclusions. The results of the current study are consistent with those of O'Shea et al. (2014) who found improvements (*vs* sham) in a simple reaction time paradigm with anodal and cathodal tDCS, but not with bihemispheric, and extends this finding to more functional tasks.

The response to anodal tDCS was found to correlate with the response to cathodal tDCS, suggesting that with mild and moderately affected patients it may not matter which cortex is targeted. The magnitude of the correlation (Pearson's R = 0.61) was consistent with that found by O'Shea et al. (2014) in their study (R = 0.6). However, this finding contrasts with Mahmoudi et al. (2011) who found a correlation between anodal and bihemispheric, but not between anodal and cathodal tDCS. Their sample size was small (n = 10) and their participants may have had higher baseline function than the current study. In the current study, neither unilateral condition correlated with bihemispheric, likely due to the overall lack of effect of bihemispheric tDCS on function, confirming a previous conclusion (O'Shea et al., 2014) that any effects of bihemispheric stimulation are not simply a sum of anodal and cathodal combined.

The reasons why bihemispheric tDCS could be less effective than unilateral are not well understood, but likely due to differences in the structures stimulated and the changes in connectivity between brain regions. Modelling studies demonstrate that current spread is dependent on the distance between the two electrodes and is therefore likely to differ between unilateral and bihemispheric arrangements. Current density is greatest below the anode for unilateral stimulation, spreading toward premotor and frontal areas which would also contribute to motor preparation. For the bihemispheric arrangement there is a medial shift of the current density, and a spread including premotor and parietal regions (Naros et al., 2016; Opitz et al., 2015). Resting state FMRI indicates different cortical network changes depending on the electrode arrangement (Lindenberg et al., 2016; Sehm et al., 2012; Sehm et al., 2013), but the relationship between change in connectivity and motor function is not yet fully understood. In the current study there were no associations between the change in JTT and TCI from the ipsilesional to the contralesional M1 for any of the electrode arrangements, making it unlikely that change in interhemispheric inhibition is responsible for the differences in response.

The hemisphere affected by the stroke appears to have an influence on the response to tDCS. The JTT improvements with tDCS were significantly greater for participants with the dominant hand affected, than the non-dominant (Figure 5.12, page 130). Similar findings have been reported for bihemispheric stimulation (O'Shea et al., 2014) and after three weeks of combined rTMS and motor practice (Ludemann-Podubecka et al., 2015). Additionally, Schade et al. (2012) demonstrated a greater magnitude of MEP facilitation with anodal tDCS when delivered to the dominant M1 compared with the non-dominant (healthy adults). This could suggest that the dominant M1 may be more susceptible to plasticity induction by electrical stimulation, which could translate into greater functional changes. This finding has implications for the delivery of tDCS in clinical practice and therefore warrants further investigation.

The location of stroke was also found to influence the response to tDCS as participants with cortical involvement demonstrated greater improvement with active tDCS than those with subcortical stroke (Figure 5.13, page 131). This is counter to some previous studies showing greater improvement for those with subcortical stroke (Hesse et al., 2011; Mahmoudi et al., 2011) and the hypothesis that subcortical stroke spares the grey matter regions that are predominantly stimulated by the tDCS. However, other studies have suggested there to be no difference in response (Lefebvre et al., 2012b; O'Shea et al., 2014). In the current study there were only eight participants with cortical involvement so the findings presented here should be interpreted with some caution. Although initial JTT was entered as a covariate in the analysis, the possibility that differences between groups for baseline function and neurophysiological characteristics (such as corticospinal excitability or GABA concentration) influenced this result cannot be discounted. Therefore, larger studies to specifically address the issue of stroke location are required.

5.5.3 No change in transcallosal inhibition or relationship with learning or function

There was no effect of tDCS on the change in iSP duration, regardless of electrode arrangement (Figure 5.15, page 133). This would appear to indicate that a single session of 1 mA tDCS of M1 is ineffective at altering the activity of interhemispheric inhibitory connections in this sample. Bolognini et al. (2011) found a decrease in TCI from contralesional to ipsilesional M1 using the dual coil approach following 10 days of bihemispheric tDCS combined with constraint of the unaffected arm. However, changes in TCI following a single session of tDCS have never been reported for stroke survivors. It is possible that the constraint of the unaffected arm is a necessary component to drive changes in TCI, but it is also possible that these changes would develop without constraint if there were repeated stimulation sessions. The TCI assessment from the contralesional M1 was only complete for 11 participants in the current study which may have been insufficient to detect an effect. Additionally, Bolognini et al. used a higher intensity for tDCS (2 mA), larger electrodes (35 cm^2) and a longer duration (40

minutes) than the current study. Their study did not find significant changes from the ipsilesional to contralesional M1, which is consistent with the current study.

There were no relationships observed between the change in iSP duration and learning or upper limb function with any active tDCS condition. Given the lack of overall change in TCI this is perhaps not surprising. Neuroimaging measures of intracortical inhibition or functional connectivity may better predict the effect of tDCS on function. For example, higher levels of ipsilesional GABA concentration have been shown to correlate with greater improvement on a simple reaction time task with anodal tDCS, explaining 86 % of the variance in response (O'Shea et al., 2014).

5.5.4 Summary and limitations

Stroke survivors with upper limb impairment demonstrated improvements in movement preparation with learning of a sequence of reaching movements with their paretic arm, but tDCS was ineffective at improving learning or the quality of their movement (speed-accuracy trade-off). There was a significant effect of electrode arrangement on within-session improvements in upper limb function, as unilateral tDCS led to small, significant, improvements in JTT performance but bihemispheric did not. However, there was no effect of tDCS on TCI, and this was not found to relate to changes in motor sequence learning or upper limb function.

It was perhaps surprising that JTT performance was improved *after* tDCS, given that learning, speed and accuracy did not change *during* tDCS. As mentioned in section 5.5.1, there are a number of potential reasons why learning could have been unaffected, including the possibility that the paradigm led to a learning strategy that was less dependent on M1 than previous tasks, and that the OT and PI measures may not have been sensitive enough to detect differences due to tDCS. It is also possible that the tDCS interacted with the motor practice (i.e.

the controlled movement of the computer mouse) to reduce inhibition within the motor cortex and improve motor control, leading to improved JTT performance which persisted after completion of the stimulation. This is consistent with the findings of Hummel et al. (2005) that JTT improvements persisted for at least 25 minutes after anodal tDCS. Changes in cortical excitability and intracortical inhibition with tDCS have also been shown to persist after the stimulation is turned off (Ardolino et al., 2005; Bastani and Jaberzadeh, 2013a; Bastani and Jaberzadeh, 2013b; Di Lazzaro et al., 2012; Kidgell et al., 2013b; Kim et al., 2014; Moliadze et al., 2014; Stagg et al., 2009). Unfortunately the JTT was not assessed during tDCS and the motor sequence learning task was not repeated at a later time, so it is unknown whether JTT improvements were evident during stimulation, or whether performance on the sequence task would have been improved after the stimulation.

There are several limitations of this study which must be considered. The sample size, although greater than many studies of this nature, may have been insufficient for the subgroup analyses and therefore the findings gained from these analyses should be interpreted with caution. Although the motor sequence learning paradigm employed allowed people to participate who were more impaired than previous studies, it was still not possible to include people with the full range of impairment seen after stroke. Therefore, the results obtained here may not hold for people with severe impairment. The use of the within-subject crossover design allowed a systematic investigation of the effect of electrode arrangement, but also meant that the study could not be conducted in the early stage after stroke when rapid changes in cortical activity and function would be taking place. Although there was no difference in response between participants who were between three and six months post-stroke and those who were more than six months post-stroke, it is possible that patients within the first three months of stroke would respond differently to the electrode arrangements. There is currently limited research at the acute stage of stroke recovery, and it is unknown whether tDCS could be of benefit as part of routine clinical practice.

Chapter 6 The effect of tDCS on retention of motor sequence learning

6.1 Abstract

Introduction: Retention of motor sequence learning may be improved by tDCS, but the influence of electrode arrangement has not been investigated.

Aims: To determine the effect of tDCS electrode arrangement on 48 hour retention of motor sequence learning in healthy adults.

Methods: Using a between-subjects study design, a cohort of 69 healthy, right handed adults (mean age 29 years, range 19- 66) were randomised to receive one of four tDCS conditions (anodal to right M1, cathodal to left M1, bihemispheric or sham) during performance of a motor sequence learning paradigm. Participants used their left (non-dominant) hand to move a computer mouse from a central square to illuminated targets on a monitor in a repeated order. TCI (iSP duration) was assessed for each hand using TMS. The trained movement sequence was repeated 48 hours later to determine the amount of learning retained.

Results: There were no differences between groups for within-session learning or retention 48 hours later. Cathodal tDCS prevented re-learning at the retention session. There was a significant increase in iSP duration from the right hand (right to left M1 TCI) irrespective of group.

Conclusions: With this explicit learning paradigm, involving gross movements of the arm, tDCS did not improve retention of learning. Therefore the effect of electrode arrangement on retention of learning is still unknown, but cathodal tDCS of left M1 affected subsequent re-learning.

6.2 Introduction

The potential of tDCS to improve learning of a motor skill over days or weeks of practice is thought to be due to facilitation of both online and offline learning effects. The M1 has been shown to play a crucial role in the consolidation of learning (Muellbacher et al., 2002). When applied to M1, tDCS has been shown to have beneficial effects on offline learning using the SVIPT, with performance improving from one day to the next with anodal tDCS, but not with sham stimulation (Reis et al., 2009). Similarly, Rroji et al. (2015) found improvements in a ballistic thumb movement task with anodal tDCS compared to sham when assessed one week later and stroke survivors with minimal levels of impairment have also demonstrated improvements in sequence learning at follow up sessions one, two and six days after training (Celnik et al., 2009). Improvements in short term (24 hour) retention have been found, using an implicit sequence learning task, with anodal tDCS of M1 (but not PMd), suggesting a key role of M1 in the retention of learning (Kantak et al., 2012). Although the exact mechanism of improvement is unknown, it is thought to be due to stabilisation or enhancement of motor memory formation, as alterations in neuronal excitability outlast the stimulation period potentially resulting in enhanced protein synthesis (Kantak et al., 2012; Reis et al., 2009).

Unilateral and bihemispheric tDCS alter cortical activity and functional connectivity differently (Lindenberg et al., 2013; Naros et al., 2016; Stagg and Johansen-Berg, 2013) and retention of motor sequence learning has not been assessed with either bihemispheric or cathodal tDCS. Therefore, the effect of tDCS electrode arrangement on retention of motor learning is unknown. If differences in retention exist then this could have implications for the use of tDCS over multiple days for rehabilitation, where skills and movements are re-learned over time and improvements between sessions would be desired. The previous tDCS studies, that have assessed retention, have utilised tasks that are predominantly implicit in nature. However, re-learning through rehabilitation requires a combination of implicit and explicit learning

strategies, and therefore the impact of tDCS on retention of explicit learning requires investigation.

This study aimed to determine:

1. the effect of tDCS electrode arrangement on 48 hour retention of motor sequence learning in healthy adults,
2. whether retention of learning was associated with changes in TCI.

The hypotheses were that:

1. retention of learning would be greater with active tDCS compared to sham,
2. better retention of learning would be associated with an increase in TCI from right to left M1.

6.3 Methods

6.3.1 Participants

Recruitment was through emails, advertisements and word of mouth between June 2013 and November 2015. Inclusion criteria were; aged > 18 years and right handed (mean laterality index 78 %, range 47 – 100 %; Oldfield, 1971). Exclusion criteria were; contraindications to TMS such as epilepsy or seizures, cardiac pacemakers or metal implants in the head. Participants denied any neurological conditions or medications that would alter central nervous system excitability. In total, 69 healthy adults, 24 male, mean age 29 years (range 19-66), completed two sessions of the motor learning task ~48 hours apart (mean 47.3, range 44-50 hours). Data from three participants had to be excluded due to problems with recording of the motor sequence learning task, leaving 66 for analysis. All experiments were approved by

the local Research Ethics Committee (BDM/11/12-35 and BDM/13/14-58) and participants provided written informed consent.

Using a between-subjects study design, participants were randomised to one of four tDCS conditions (sham, anodal, cathodal or bihemispheric) using a random number generator. Characteristics of the participants in each group are in Table 6.1, page 152.

6.3.2 Paradigm

The motor sequence learning task was performed as described in Chapters 3 and 4. Briefly, participants repeated a sequence of 12 movements, 25 times, with their non-dominant (left) hand. This hand was chosen as the use of a computer mouse with the non-dominant hand was novel and considered to be difficult, whereas all of the participants were familiar with the use of a computer mouse with their dominant hand.

6.3.2.1 Session one

Participants first completed two practice sequences to familiarise them with the movement of the mouse to the targets. They were then informed that they would repeat the same sequence of 12 movements, 25 times, and that they could anticipate target appearance if they knew which would be next. The sequence for each participant was chosen randomly from a pool of 8 sequences. Following completion of the 25 repetitions of the sequence, two random sequences (12 movements each) were performed to distinguish between general learning and sequence specific learning effects. Finally, one additional repetition of the trained sequence was completed to ensure that the participants left the laboratory with the trained sequence being the last one performed, rather than a random sequence.

6.3.2.2 Session two

Participants completed three repetitions of the trained sequence to determine the amount of learning retained at 48 hours. Participants were not informed that they would be performing the same sequence again until they reached the laboratory, in an attempt to minimise potential differences between participants in active attempts to remember the sequence.

6.3.3 Stimulation of primary motor cortex

6.3.3.1 Setup

In session one only, TMS was used to determine the position of each M1 for placement of the tDCS electrodes, and to assess TCI at baseline and immediately post-stimulation.

Muscle activity (EMG) was recorded from each FDI and processed as specified in Chapter 4. A figure-of-eight coil (70 mm diameter) was used with a Magstim 200^2 Bistim stimulator (Magstim Company, UK) to elicit MEPs, while participants rested their hands prone on a pillow on their laps. The optimal position for evoking MEPs in the relaxed FDI was established and marked with a water-soluble marker directly on the scalp to ensure consistent coil placement.

6.3.3.2 Transcallosal inhibition

A TMS intensity of 80 % MSO was used to asses TCI as specified in Chapter 4. Briefly, participants were instructed to activate their FDI at ~ 75 % of their maximal effort while single pulse stimuli were delivered to the ipsilateral M1. Twenty stimuli were delivered to each M1 before and immediately following performance of the motor sequence learning task. Each trace was rectified then an average waveform constructed. The duration of TCI was calculated for the average trace from the time where the EMG activity dropped below 75 % of the pre-stimulus level to when it returned above this level.

6.3.3.3 Transcranial direct current stimulation

The tDCS was delivered in session one only using a constant current stimulator (Mind Alive, Canada or NeuroConn, Rogue Resolutions, UK), for 20 minutes at 1 mA during the motor sequence learning task. As described in Chapter 4, for anodal tDCS the anode was placed over the right M1 at the hotspot for FDI and the cathode over the contralateral orbit, for cathodal tDCS the cathode was placed over the left M1 (ipsilateral to the performing hand) and the anode over the contralateral orbit, and for bihemispheric tDCS the anode was placed over right M1 and the cathode over left M1 (see Figure 4.2, Chapter 4). Sham tDCS was delivered in a standard manner, in either of the electrode arrangements (randomly assigned). Participants were blinded as to whether they received active or sham stimulation, and the experimenter who was in the room with the participant during performance of the task was also blinded.

6.3.4 Analysis

The OT for each target was recorded automatically by the Matlab programme into an Excel spreadsheet for offline analysis. As described in Chapter 3, the median OT was calculated for each of the repetitions, normalised to the first repetition, and averaged across consecutive repetitions to form 13 blocks and 3 retention repetitions (R1 – R3).

Based on previous studies which have demonstrated large effects of active tDCS on retention of learning (Kantak et al., 2012; Lefebvre et al., 2015; Rroji et al., 2015), it was estimated that the effect size would be at least 1.0. Therefore, it was determined that 17 participants *per* group would be required in order to find a difference at the retention test between active and sham stimulation with $\alpha = 0.05$ and power of 80 %. Statistical analyses were conducted using SPSS 21.0 (IBM Inc.). Normality was assessed using Kolmogorov-Smirnov tests and visual inspection of frequency histograms. Due to a wide range in age for each group, age was entered as a covariate for each analysis of learning and retention. Violations of sphericity were

corrected using the Greenhouse-Geisser correction and significance was set at $p < 0.05$. Data are presented as mean ± SEM unless otherwise specified.

6.3.4.1 Participant characteristics

To assess differences in participant characteristics between groups, independent samples Kruskal-Wallis tests were used for age and handedness and one-way ANOVAs were used for initial OT and time between sessions (hours). A Chi square test was used to assess differences in the number of males and females in each group.

6.3.4.2 Online learning

A 12 BLOCK × 4 GROUP mixed rmANOVA was used to test for changes in normalised OT over the learning blocks and for the effect of tDCS condition (GROUP). Specificity of sequence learning was assessed using a 2 BLOCK × 4 GROUP mixed rmANOVA with the normalised OT of the last block of the repeated sequence and the subsequent random block.

6.3.4.3 Retention of learning

The effect of tDCS on retention of learning was assessed using a 2 TIME × 4 GROUP mixed rmANOVA with the normalised OT of the last block of the repeated sequence (block 13) and the first repetition of the retention session (R1).

Re-learning (i.e. change in OT) during the retention session was assessed using a 3 RETENTION REPETITION × 4 GROUP mixed rmANOVA with the normalised OT of each of the three repetitions in the retention session. For the interaction effect, paired samples t-tests were used to compare normalised OT from the first (R1) and third (R3) repetition for each group separately.

6.3.4.4 Transcallosal inhibition

A one-way ANOVA was used to determine whether there was any difference between groups for baseline TCI (iSP duration) from each hemisphere separately. Hemispheric asymmetry in TCI was assessed using a paired t-test comparing baseline iSP duration from each FDI. A 2 TIME × 4 GROUP mixed rmANOVA was used to assess for differences in TCI duration between baseline and post-stimulation.

The relationship between the change in TCI duration and retention of learning (normalised OT difference between the last block of the repeated sequence and the first repetition of the retention session) was assessed using a Pearson correlation with all participants grouped together.

6.4 Results

6.4.1 Participant characteristics

There were no differences between groups for age (p = 0.88), handedness (p = 0.94), initial absolute OT (i.e. reaction to target illumination, p = 0.65) or the time (hours) between the two sessions (p = 0.64). Chi-square test showed no group differences for sex (p = 0.2). Group characteristics are presented in Table 6.1.

Table 6.1 Group characteristics.

	n	**Age** *(years)* mean (SD)	**Handedness** *(%)* mean (SD)	**Sex** male n (%)	**Initial OT** *(s)* mean (SD)	**Hours between sessions** mean (SD)
Sham	16	27.6 (7.9)	76.8 (17.5)	8 (50.0)	0.37 (0.03)	47.3 (0.6)
Anodal	17	29.1 (11.1)	79.7 (18.0)	5 (29.5)	0.37 (0.04)	47.5 (0.5)
Cathodal	16	28.1 (7.7)	79.0 (17.9)	7 (43.8)	0.35 (0.08)	47.3 (0.9)
Bihemispheric	17	29.4 (11.8)	78.3 (15.9)	3 (17.6)	0.36 (0.04)	47.2 (1.0)

6.4.2 Change in OT over the blocks

The 12 BLOCK × 4 GROUP mixed rmANOVA with AGE as a covariate revealed an effect of BLOCK ($F_{5.3,323.4}$ = 21.413, p < 0.001) but no effect of GROUP ($F_{3,61}$ = 0.810, p = 0.493), no interaction between BLOCK and GROUP ($F_{15.9,323.4}$ = 1.133, p = 0.323; Figure 6.1) or between BLOCK and AGE ($F_{5.3,323.4}$ = 1.568, p = 0.165). This indicates that OT reduced with training of the repeated sequence across all groups.

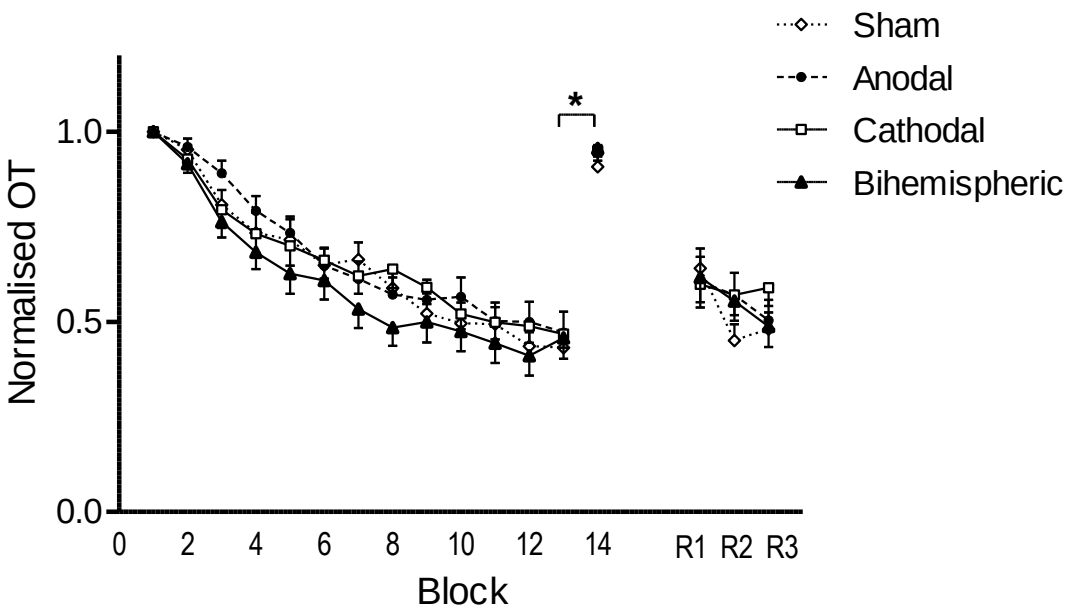


Figure 6.1 Normalised OT for each group (mean ± SEM).
Block 14 represents random block, R1-3 = retention repetitions 48 hours later. There was a significant effect of block as the normalised OT reduced with training. * significant difference between last repeated block and random block (averaged across groups, p < 0.001). There was no significant difference between the first retention repetition (R1) and the last block of the repeated sequence from session one (p = 0.250).

6.4.3 Specificity of sequence learning

The last block of the repeated sequence differed significantly from the random block (effect of BLOCK: $F_{1,61}$ = 58.437, p < 0.001) but there was no interaction with GROUP ($F_{3,61}$ = 0.059, p = 0.981) or with AGE ($F_{3,61}$ = 2.974, p = 0.090) indicating that reductions in OT were specific to the trained movement sequence (Figure 6.1).

6.4.4 Retention of learning

The last block of the repeated sequence did not differ from the first repetition of the retention session (effect of BLOCK: $F_{1,61}$ = 1.351, p = 0.250) suggesting that the improvements in OT were retained 48 hours later. There was no interaction with GROUP ($F_{3,61}$ = 0.509, p = 0.678) or AGE ($F_{3,61}$ = 0.656, p = 0.421) indicating that retention was not dependent on tDCS condition (Figure 6.1).

There was a significant interaction between RETENTION REPETITION and GROUP ($F_{4.2,85.9}$ = 2.464, p = 0.048) as re-learning was dependent on the tDCS condition received during the training session. Normalised OT reduced from the first (R1) to the third (R3) retention repetition for sham (p = 0.026), anodal (p = 0.009) and bihemispheric (p = 0.016) groups but not for the cathodal tDCS group (p = 0.863, Figure 6.2).

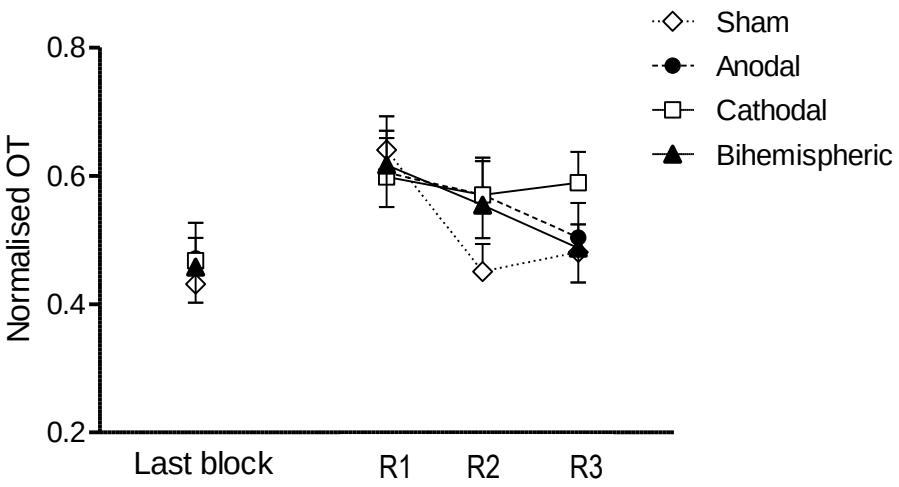


Figure 6.2 Normalised OT for each tDCS condition for retention session.
Last block of training session is shown for information. R1-3 = retention trials 48 hours later. Re-learning was dependent on group (group by repetition interaction) as there was no change between R1 and R3 for cathodal tDCS only (p = 0.863).

6.4.5 Transcallosal inhibition

There was no effect of GROUP on baseline iSP duration from either hand (left FDI: $F_{3,65} = 0.399$, $p = 0.754$; right FDI: $F_{3,65} = 2.105$, $p = 0.109$). Paired t-tests indicated no difference in iSP duration between right and left hands, indicating no hemispheric asymmetry in TCI.

For iSP duration from the left FDI (left to right M1 TCI) there was no effect of TIME ($F_{1,62} = 0.379$, $p = 0.541$) or GROUP ($F_{3,62} = 0.521$, $p = 0.583$) and no interaction ($F_{3,62} = 0.654$, $p = 0.583$) indicating that TCI duration did not change. For iSP duration from the right FDI (right to left M1 TCI) there was an effect of TIME ($F_{1,62} = 17.606$, $p < 0.001$) as TCI duration was longer post-stimulation (grand mean: 27.5 ± 0.9 ms) than the baseline (25.0 ± 0.9 ms). However, there was no effect of GROUP ($F_{3,62} = 1.547$, $p = 0.211$) or interaction ($F_{3,62} = 0.812$, $p = 0.492$) indicating that this was not due to the tDCS condition(Figure 6.3).

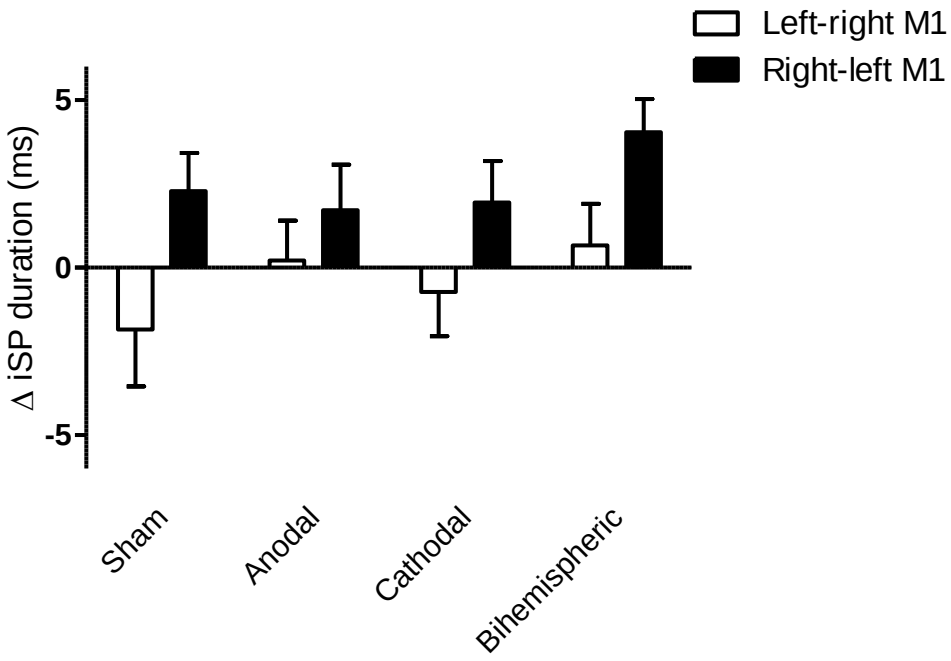


Figure 6.3 Change in TCI (iSP duration) from each hemisphere for each tDCS condition (mean ± SEM). There was no effect of group or interaction with time for either hemisphere. When pooled across groups there was an increase in iSP duration from the right hand post-stimulation (right to left M1 TCI; $p < 0.001$).

When all participants were grouped together there were no correlations between the change in iSP duration from either hand and the OT difference between the last block of session one and the first repetition of the retention session (left to right M1 TCI: R = -0.179, p = 0.150, right to left M1 TCI: R = 0.016, p = 0.897). See Figure 6.4.

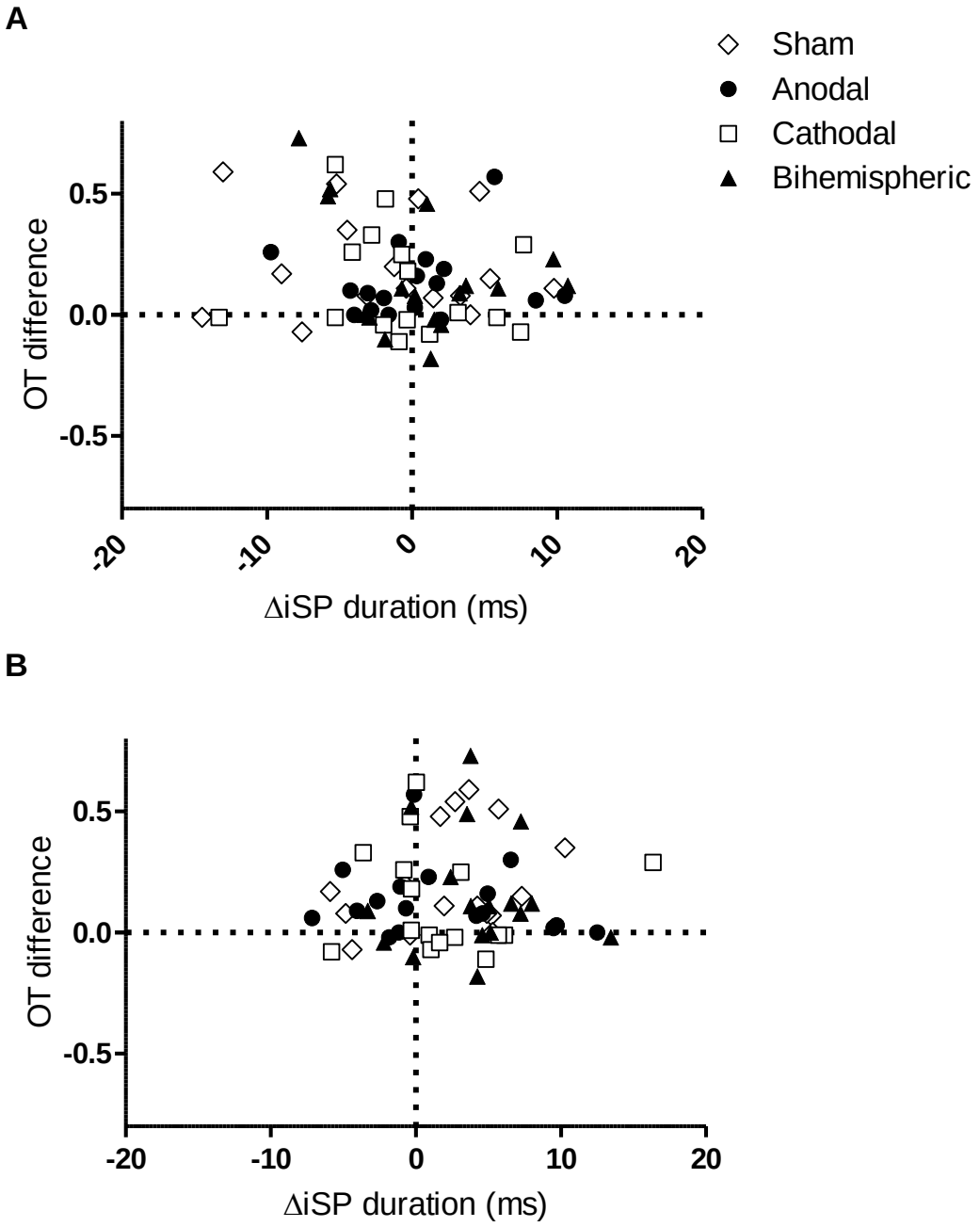


Figure 6.4 OT difference between sessions as a function of change in iSP duration for each group. **A.** iSP from left FDI (left to right M1 TCI), **B.** iSP from right FDI (right to left M1 TCI). There were no correlations for either hemisphere.

6.5 Discussion

The main finding of this study was that tDCS did not significantly alter online motor sequence learning or 48 hour retention. However, cathodal tDCS delivered to the left M1 during the training session did lead to an impairment in re-learning (i.e. reduction in OT) over the three repetitions of the retention session. Right to left M1 TCI was found to increase following performance of the motor sequence learning task with the left hand but this was independent of tDCS condition, suggesting that tDCS did not alter inhibition between motor cortices. There were no relationships between changes in TCI from either hemisphere and retention of OT improvements, indicating that in healthy adults a change in TCI does not underlie the formation of motor memories in order to retain knowledge of a sequence of movements.

6.5.1 No effect of tDCS on within-session motor sequence learning

The lack of improvement in online motor sequence learning with active tDCS compared to sham is consistent with the findings from the crossover study using the same paradigm with healthy young and older adults (Chapter 4). Changes in OT were specific to the trained sequence, but specificity of learning did not depend on tDCS condition. As discussed in Chapter 4, the participants may have utilised a learning strategy that did not depend on M1 and therefore tDCS to alter activity of M1 had no effect on learning with this task. Alternatively, the tDCS may have been insufficient to alter cortical activity in this healthy sample. Although the stimulation parameters were similar to those used previously to assess motor learning, there was no alteration of inhibition between hemispheres due to tDCS (Figure 6.3, page 155), possibly suggesting that the stimulation parameters were not optimal. However, In Chapter 4 a significant increase in TCI from right to left M1 was observed for young adults with bihemispheric tDCS using the same stimulation parameters, which was not seen in the current study. This may be due to the variability between subjects in the current study as this was a between-group subject design. A crossover study design (as used for Chapter 4) may minimise

variability by delivering all tDCS conditions to the same individuals. Variability between subjects is commonly reported in studies of non-invasive brain stimulation and could be due to individual differences in anatomy, functional connectivity between brain regions and capacity for alterations in cortical excitability. This will be discussed further in Chapter 7.

6.5.2 No effect of tDCS on retention of motor sequence learning

Performance improvements were maintained when assessed 48 hours later, indicating that the participants were able to consolidate their learning. Previous studies have found that performance is better after a consolidation period if participants received anodal tDCS in comparison with sham (Kantak et al., 2012; Reis et al., 2009). In contrast, in the current study, tDCS did not influence the retention of the learned sequence (Figure 6.1, page 153) assessed 48 hours later. Differences may be due to the nature of the tasks and the stimulation parameters. Kantak et al. (2012) compared anodal tDCS of M1 with PMd and demonstrated that M1 stimulation significantly improved both within-session implicit learning and retention the next day. However, the current density was higher (0.125 $mA.cm^{-2}$) than the current study (0.04 $mA.cm^{-2}$). Improvements in SVIPT performance have been demonstrated when tDCS is applied over multiple days of practice (Reis et al., 2009) which was due to improvements in offline learning rather than improvements within the training session. Both of these tasks (Kantak et al., 2012; Reis et al., 2009) tested implicit motor learning, whereas the current task was an explicit sequence learning paradigm. To this authors knowledge the effect of tDCS on the retention of explicit motor sequence learning has not been studied previously, but the current results would suggest that it does not improve retention of learning with the non-dominant (left) hand. This may indicate that the formation of explicit memory may be independent of M1.

Consistent with the findings of Reis et al. (2009), Rroji et al. (2015) found that although anodal tDCS did not improve within-session performance of a ballistic thumb flexion task, retention

measured one week later was improved. However, this improvement was not evident at the retention test the next day, suggesting that the timing of the retention test is an important factor to consider.

In the study by Rroji et al. (2015) three blocks of the task were performed during the first follow up session, leading to the possibility that either tDCS improved long term retention only, or that the tDCS interacted with the practice the next day to influence retention one week later. The current study found that, although active tDCS did not affect retention of learning, cathodal tDCS applied to left M1 during the training session significantly impaired the re-learning during the retention session. Similarly, Richardson et al (2006) demonstrated reduced re-learning of an adaptation task when participants had received 1Hz repetitive TMS (rTMS) to disrupt M1 prior to the initial learning period. It is likely that participants developed a strategy for learning with the current task during the first session and could therefore re-learn rapidly when exposed to the same movement sequence again. This is supported by the findings from Chapter 3 that learning is improved following the first exposure to the task and is likely to be similar to the concept of "savings" reported with visuomotor adaptation and perturbation tasks (e.g Huang et al., 2011; Kitago et al., 2013; Leow et al., 2014). It is therefore tempting to speculate that reducing activity of the motor learning network through reduction in left M1 excitability prevents participants from consolidating this skill leading to impaired re-learning in the next session. Leow et al. (2014) found that "savings" were not improved by anodal tDCS of M1, but did not assess cathodal stimulation. Further studies should investigate whether similar results are observed with cathodal stimulation of right M1 or when performing the current paradigm with the right hand to determine the conditions under which this impairment is seen.

6.5.3 No relationship between change in TCI and retention of learning

There was no relationship between change in TCI and retention of OT improvements, suggesting that change in inhibition between hemispheres is an unlikely mechanism underlying retention of motor sequence learning. However, given that tDCS did not alter retention of learning, or TCI duration overall, this possibility cannot be discounted completely. Changes in GABA have been associated with improvements in function after stroke (Blicher et al., 2015) and could therefore be a more likely mechanism underlying retention of learning. Additionally, people with higher grey matter volume in the dorsolateral prefrontal cortex and SMA have been found to be able to retain acquired motor skills for longer than people with lower volumes (Sampaio-Baptista et al., 2014) and therefore a between-group study design may not have been completely appropriate if variations between participants were present.

When averaged across tDCS conditions, TCI duration from right to left M1 was found to increase after the motor sequence learning task. This is likely due to the enforced use of the non-dominant (left) hand during the motor learning task which could have led to an increase in right M1 excitability. The interhemispheric imbalance model (for stroke recovery) would suggest that this would therefore lead to greater inhibition passed across to the dominant hemisphere, which was indeed the case. To this author's knowledge no studies have demonstrated changes in TCI with motor learning tasks alone. Changes in TCI or the balancing of hemispheric asymmetry in cortical excitability underlies the rationale for constraint induced movement therapy as a technique for improving stroke rehabilitation. The non-paretic limb is constrained to force the patient to utilise their affected arm for motor practice and activities of daily living. Although the current study did not constrain the dominant hand, it was not involved in the performance of the motor sequence learning task, and as such remained still, on the persons lap, throughout that portion of the experiment. TCI measures post-stimulation were taken immediately so there was little chance for the participant to use their dominant hand between completion of the task and assessment of TCI changes.

6.5.4 Summary and limitations

Overall this study suggests that tDCS does not affect 48 hour retention of an explicit sequence learning task, but that cathodal tDCS of the dominant (left) M1 may impair subsequent re-learning with the non-dominant hand two days later. This study had an adequate sample size, and there were clearly no differences between groups for most measures, making it unlikely that null results can be explained by a lack of statistical power. However, it is possible that the task was not challenging enough for healthy adults to demonstrate improvements with active tDCS compared to sham. Additionally, the learning and remembering of the movement sequence may have relied on spatial working memory and plasticity of the hippocampus, rather than motor learning involving M1. If this is the case then it is unlikely that M1 stimulation would have a measurable effect. Future studies should examine whether changes in TCI and potentially GABAergic inhibition relate to retention of learning using a more challenging task that is known to be influenced by tDCS.

Chapter 7 General Discussion

This thesis investigated the influence of electrode arrangement on neuromodulation with tDCS in healthy ageing and in people with neurological impairment due to stroke. A motor sequence learning paradigm was developed that could be utilised by all groups of participants, to systematically compare learning between anodal, cathodal and bihemispheric electrode arrangements. The experiments in Chapter 3 described and tested the novel sequence learning paradigm. Healthy adults and stroke survivors were found to improve their reaction to target illumination (OT) over 25 repetitions of a movement sequence, with a similar pattern of changes as those observed with the common "key press" sequence learning paradigms. The learning measure was sensitive enough to detect impairment in sequence specific learning for stroke survivors with upper limb impairment in comparison with healthy, age-matched controls. The remaining studies therefore utilised this paradigm. There was no effect of tDCS on motor sequence learning or retention found in the remaining chapters, regardless of electrode arrangement, but clear differences in learning were found between young and older adults. Despite the lack of improvements with tDCS for this experimental learning paradigm, there was an effect of tDCS electrode arrangement on JTT performance for stroke survivors, with improvements following unilateral, but not bihemispheric, tDCS.

7.1 Motor sequence learning

The studies in Chapters 4, 5 and 6 sought to determine whether the electrode arrangement used for tDCS delivery would impact on changes in motor sequence learning performance in healthy adults and people with upper limb impairment after stroke. In all of these studies a null result was found, indicating that active tDCS over M1 did not improve motor sequence learning, regardless of whether unilateral (anodal or cathodal) or bihemispheric tDCS was used. This was unexpected, as it was hypothesised in all studies that tDCS would increase cortical excitability to aid plasticity and improve the rate or amount of learning. Based on the

interhemispheric imbalance model, it was anticipated that bihemispheric tDCS would provide additional benefit over unilateral by increasing the excitability of the M1 under the anode directly and also indirectly due to decreases in transcallosal inhibition from the opposite M1 (site of the cathode).

Motor sequence learning performance was chosen as the outcome measure across all studies, rather than a more functional measure such as the JTT. This was, at least in part, so that the rate of change in performance during stimulation could be assessed in additional to the total change. It was thought possible that tDCS may not improve the performance level that participants reach, but rather the amount of training needed to reach that level. Therefore, a number of variables were assessed throughout. The pattern of change in OT or PI over the 13 blocks could distinguish differences in the rate of learning as a tDCS by block interaction, the AUC provided a single value for analysis which takes into account both the rate and the amount of change over the blocks, and the sequence specific learning measure assessed the total learning at the end of training relative to an untrained sequence. Additionally, the number of anticipations of target appearance was recorded as an explicit learning measure. These variables were sensitive enough to exhibit group differences between young and older adults but none of these variables, nor the retention of OT improvements, revealed an effect of tDCS. The lack of effect of tDCS on online learning was consistent whether a within-subject (Chapter 4) or between-subject (Chapter 6) study design was used.

There are numerous potential explanations for the lack of effect and it is likely that a combination of reasons influenced this result. The gross arm movements, the explicit nature of the task, and the provision of a familiarisation session likely reduced the difficultly of the task and increased the rate of change in OT in comparison with the SRTT (Nissen and Bullemer, 1987). This may mean that there was less capacity to detect improvement with tDCS. However, the validity of this explanation is limited by the findings that both the older adult group and

the stroke group showed some impairment in performance, but still did not show improvement with tDCS. In the studies with healthy adults (Chapters 4 and 6), the non-dominant (right) M1 was the focus for the stimulation and the task was performed with the non-dominant hand. This choice could have limited the efficacy of the tDCS if the left M1 is primarily responsible for the control of motor sequence learning, as it is for motor imagery (Fadiga et al., 1999; Nair et al., 2003; Stinear et al., 2006) and the control of fine finger movements (Kim et al., 1993). Additionally, greater facilitation of MEPs with tDCS has been reported for the dominant M1 in comparison with the non-dominant (Schade et al., 2012). The findings of the JTT subanalyses (Chapter 5) showed greater improvement with active tDCS for the group with the dominant hand affected. Similar findings have been presented following three weeks of rTMS to the contralesional M1 (Ludemann-Podubecka et al., 2015). This raises the possibility that the dominant M1 is more adaptable in response to external stimuli and perhaps better able to modulate neurotransmitter concentration or more susceptible to increases in excitability. However, this is speculation and requires further investigation. Unfortunately a consistent lack of tDCS effect across studies makes it impossible to draw conclusions as to whether any of the electrode arrangements is preferential to another for improving the learning of movement sequences.

There are inconsistent conclusions throughout the literature as to whether tDCS can improve motor sequence learning ability, with some studies showing improvements (Kantak et al., 2012; Naros et al., 2016; Nitsche et al., 2003c; Stagg et al., 2011; Vines et al., 2008; Zimerman et al., 2013), but others showing no effects (Amadi et al., 2015; Ambrus et al., 2016; Kang and Paik, 2011) and mixed results reported in reviews (Hashemirad et al., 2016; Savic and Meier, 2016). The possible reasons for these mixed results extensive, and have been discussed throughout the previous chapters. Briefly, likely factors include differences in stimulation parameters, the difficulty or the accuracy requirements of the learning tasks, demographics of the participants, the hemisphere stimulated, the nature of the learning (explicit *vs* implicit) and

the variability in response to tDCS in terms of the change in cortical excitability. If tDCS is in fact effective at improving motor sequence learning, then the variation in results would suggest that the effect is very small and variability between and within subjects may be greater than the effect of the stimulation. Therefore, studies with large samples would be required to determine whether differences in response occur across the different electrode arrangements. Whether any changes would have clinical significance remains to be seen.

7.1.1 No relationship between changes in motor sequence learning and TCI

The studies in Chapters 4 and 5 also aimed to test for potential relationships between changes in motor sequence learning with tDCS and those in TCI, to determine whether alteration of TCI could be responsible, at least in part, for any improvements in function observed with tDCS. Although a small, but significant, increase in iSP duration was evident from the right M1 (site of the anode) for the younger (healthy) group after bihemispheric tDCS in Chapter 4, there was no reduction in TCI from the opposite M1 (site of the cathode) as would be expected based on the rationale for delivering tDCS to both motor cortices simultaneously. There was no effect of tDCS for the older adults (Chapter 4) or the stroke survivors (Chapter 5). The results of the correlation analyses indicated no relationships for any active tDCS condition between changes in TCI and learning (Figure 4.13, 4.14, 5.16) or JTT performance (Figure 5.17). These findings combined would appear to indicate that a single session of tDCS at best has a minimal effect on TCI, particularly in older adults, and that changes in interhemispheric inhibitory connections between cortices do not underlie functional gains with tDCS.

However, iSP duration is just one measure to assess transcallosal inhibition. The paired pulse (dual coil) method may produce different results as this technique assesses reductions in corticospinal excitability through a suppression of MEPs, rather than the interruption of voluntary muscle activity. Indeed, previous studies using the dual coil technique have reported changes following a single session (Tazoe et al., 2014), or repeated sessions in combination

with constraint of the arm (Bolognini et al., 2011). The iSP measure was chosen for this study, rather than the dual coil method, partly due to logistical considerations with only one experimenter, and also because it is thought to better reflect changes in voluntary motor activity (Giovannelli et al., 2009). Changes in TCI are likely to be dependent on changes in both glutamatergic neurons and GABAergic interneurons, and there is still limited understanding of precisely what the iSP is measuring. Future studies using multiple methods of assessment are required to further understand the effect of tDCS on changes in TCI. Assessments should include TMS techniques (iSP and dual coil), MRI to examine changes in resting functional connectivity between cortices, DTI to examine the structure of the corpus callosum and MRS to test for changes in GABA and glutamate concentration. This would enable progress in our understanding not only of how these measures change with tDCS, but how changes in each of these measures relate to another.

7.1.2 Speed-accuracy trade-off

There were inconsistent findings across studies with regard to changes in speed and accuracy with learning of the movement sequence. In Chapter 3, healthy younger adults demonstrated significant, sequence specific, increases in speed of cursor movement with learning, but healthy older adults and stroke survivors did not. As discussed in Chapter 3, it may be that younger adults found it easier to learn the movement sequence and could thus divide their attentional resources to improve movement speed alongside improvements in OT. Older adults are reported to have reduced ability to do two tasks at once (dual-tasking) and may over-activate brain regions in order to preserve performance on a cognitive or motor task (for review see; DeCarli et al., 2012; Reuter-Lorenz and Lustig, 2005). There was no change in accuracy for any of the groups and so the use of a performance index, which takes into account the balance of changes in both speed and accuracy of movement, was justified for Chapters 4 and 5. This performance index measure has been used previously for a motor

learning task requiring movement of a computer mouse around a circuit (Lefebvre et al., 2012a; Lefebvre et al., 2012b).

In Chapter 4, an improvement in the performance index was seen, without an effect of tDCS, age group or any interaction. This indicated that both older and younger adults improved speed and/or accuracy of movement, without sacrificing the other, alongside learning of the movement sequence. However, this was not found to be specific to the trained movement sequence and was therefore a general effect of task practice within the session rather than related to the learning of the specific sequence of hand movements. Nevertheless, this study was the first to demonstrate that improvements in the performance index measure could be observed with a sequence learning paradigm that is similar in concept to the SRTT, but that these changes appear to be unaffected by tDCS to M1.

Although healthy adults demonstrated improvements in PI with training, there was no such finding for the stroke survivors, regardless of tDCS condition (Chapter 5). This may be related to the use of the paretic arm to perform the task. It may be that the motor system was operating at capacity in order to perform the task and learn the movement sequence, leaving little room for improvements in the control of their movements.

It was surprising that tDCS had no effect on the PI for either study (Chapter 4 or 5), as Lefebvre et al. (2012b; 2015) demonstrated improvements in PI with their circuit learning task with bihemispheric tDCS, and it was expected that the current study would demonstrate similar effects. They utilised larger electrodes (35 cm^2 *vs* 25 cm^2) and a longer duration of stimulation (30 minutes *vs* 20 minutes), raising the possibility that either the stimulation duration chosen here was insufficient to produce reliable improvements or that the larger electrodes used by Lefebvre et al. stimulated additional motor regions (such as PMC) which contributed to the improved motor control. Indeed, improved performance one week after bihemispheric tDCS

was associated with altered ipsilesional dorsal premotor cortex activity (Lefebvre et al., 2015). The PMC is thought to play a role in motor preparation, including the control of direction and speed of movement (for review see; Hoshi and Tanji, 2007).

7.2 Impaired sequence specific learning for stroke survivors

The studies of Chapters 3 and 5 demonstrated that stroke survivors with upper limb impairment were capable of learning a movement sequence with their paretic arm, but in Chapter 3 there was also evidence of reduced sequence specific learning in comparison with age-matched controls. This study was the first to demonstrate this impairment, which could have implications for re-learning of everyday tasks through rehabilitation, and therefore warranted confirmation with another sample of stroke survivors.

The data from the sequence specific learning measure (difference in normalised OT between the last block of the repeated sequence and the random block) were compared between healthy adults (Chapter 4) and stroke survivors (Chapter 5). All healthy adults used their non-dominant (left) hand and stroke survivors used their paretic arm to complete the task. Appendix F and Figure 7.1 (below) present the details and results of this comparison, which confirmed that stroke survivors demonstrate impaired sequence specific learning with their paretic arm in comparison with age-matched healthy adults, irrespective of tDCS condition. Motor learning underlies rehabilitation strategies such as task specific training (Hubbard et al., 2009). Therefore, this finding suggests that stroke survivors show reduced capacity for learning of sequential movement patterns which are common in everyday life. It may be that they require a higher number of repetitions of a movement sequence in order to learn it effectively, but this may be difficult to achieve with limited time for focused rehabilitation as part of routine clinical practice, and fatigue could accompany increased motor practice. Robot therapy, which would allow a larger number of repetitions to be performed in a session, is

under investigation throughout the rehabilitation literature, but is beyond the scope of this thesis.

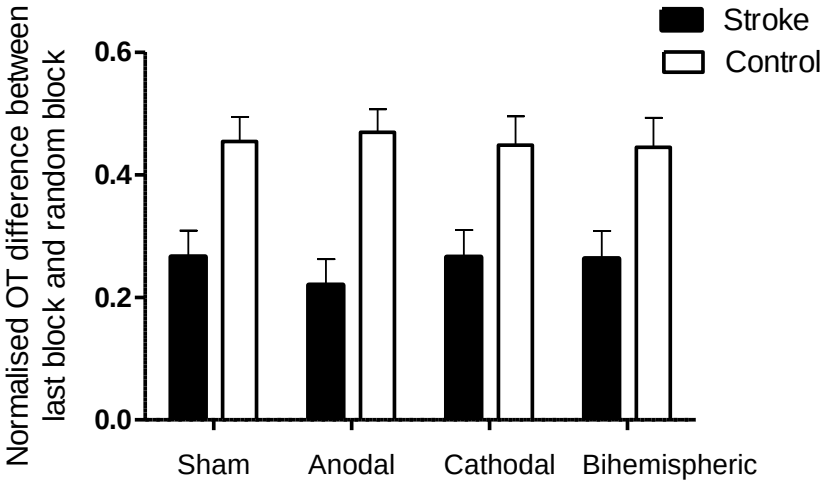


Figure 7.1 Sequence specific learning for stroke survivors and control groups.
Stroke survivors demonstrate impaired sequence specific learning across all tDCS conditions.

7.3 Advantages and limitations of the sequence learning paradigm

The sequence learning paradigm was developed as part of these studies to be an explicit learning task, rather than testing implicit motor sequence learning as some other studies have done. Participants were specifically informed of the presence of a repeated sequence of movements and encouraged to learn the movement sequence and anticipate target appearance if they could. This choice was made for a number of reasons. Firstly, since it was developed with the intention of being utilised repeatedly in the same participants over several weeks it was thought that if used as an implicit learning task then some participants might become aware of the repeating sequence in one of their sessions, which could lead them to take a different approach to the task in the next session. This could therefore invalidate results if some sessions were "implicit learning" whereas others became "explicit" unintentionally. Secondly, rehabilitation of movement most likely requires elements of both implicit and explicit learning and therefore an understanding of the effect of stroke on explicit sequence learning is needed.

There were several different measures taken from this paradigm and improvements with training were likely due to a combination of implicit and explicit learning. For OT, participants could actively anticipate target appearance which is essentially explicit in nature, but they could also subconsciously attend preferentially to the target which they implicitly knew would light up next, and this could result in a quicker reaction time. Changes in the speed accuracy trade-off are also likely to have elements of implicit learning as the main focus of the task was on the time to leave the central square. However, the use of an explicit learning task also comes with limitations. Attention levels may vary between participants and across sessions which could lead to variability in performance. Additionally, some participants may be less confident in their own abilities to learn in comparison with others and therefore nervous about anticipating target appearance even if they think they know which target will be next. The use of the within-subject crossover design would have helped to reduce the impact of this limitation, but it still requires consideration.

7.4 Variability in the response to tDCS

Variability in the response to non-invasive brain stimulation is commonly reported throughout the literature and a number of studies have systematically investigated variability in MEP changes with tDCS between and within subjects (Dyke et al., 2016; Horvath et al., 2016; Lopez-Alonso et al., 2014; Strube et al., 2016; Wiethoff et al., 2014). This variability no doubt increases the likelihood of studies producing null results as approximately half of subjects do not show the expected response.

The studies in this thesis demonstrated considerable variability in the functional response (i.e. learning) to tDCS for healthy participants. The goal of all active conditions was to increase excitability of the right M1 and improve performance of the left hand but only 24 % showed improvement in the OT AUC across all active conditions (anodal, cathodal and bihemispheric) in comparison with sham, 33 % showed a mixed response (some improvements, some

performance decrements), and the remainder showed worse performance for all active conditions. This variability is likely to have contributed to the lack of overall effect of tDCS on learning, in addition to the potential reasons with regard to the nature of the learning paradigm that have been discussed in previous sections. The stroke group also demonstrated variability, with the majority of participants having a mixed response across active tDCS conditions for both OT AUC (63 %) and JTT change (54 %). Only 13 % showed improved OT AUC across all active conditions, and 38 % showed improved JTT performance across all active conditions. There was a significant correlation for JTT improvements between anodal and cathodal conditions, suggesting better consistency if bihemispheric tDCS was not included.

The variability in the response to tDCS is not yet understood. Studies attempt to account for, or even control, factors such as time of day, sleep, attention, pre-stimulation activity and menstrual cycle, but variability persists and results can be unclear. This raises the importance of conducting large scale studies, as well as systematic reviews and meta-analyses to improve our understanding of the effect of tDCS on the motor system. If we can better predict who will and will not respond to tDCS then it is more likely to have a chance of being used routinely for improving function in ageing and neurological conditions such as stroke.

7.5 Summary of findings

Overall this thesis presented novel findings which contribute to our understanding of motor control and neuromodulation through tDCS as well as raising further research questions. A summary of the main findings is below:

1. A novel paradigm involving a repeated sequence of gross hand movements led to improvements in motor preparation with a similar pattern of change as other sequence learning tasks involving key presses with individual fingers.
2. Stroke survivors with upper limb impairment were capable of improving their motor preparation with training of a movement sequence with their paretic arm.
3. Stroke survivors showed reduced sequence specific learning in comparison with healthy, age-matched controls. This impairment was not improved by delivering tDCS to M1.
4. Healthy older adults showed reduced motor sequence learning ability with their non-dominant hand in comparison with younger adults. This impairment was not improved by delivering tDCS to M1.
5. Healthy young and older adults, but not stroke survivors, demonstrated improvements in the speed-accuracy trade-off with learning of a sequence of movements.
6. Active tDCS did not improve the learning of a sequence of gross arm movements, regardless of electrode arrangement.
7. There was minimal effect of tDCS on TCI, assessed as the change in iSP duration.
8. Anodal or cathodal tDCS improved JTT performance for stroke survivors with mild and moderate upper limb impairment, but bihemispheric tDCS was ineffective.
9. The hand affected by the stroke and the location of the stroke had an impact the response to tDCS.
10. Cathodal tDCS delivered during training led to impaired re-learning 48 hours later for healthy adults.

The finding with the most obvious implications for rehabilitation was that of an effect of electrode arrangement on JTT improvements, leading to the possibility that unilateral tDCS may be more effective as an adjuvant to rehabilitation. Anodal tDCS has recently been demonstrated, in a comprehensive study, to effectively improve recovery of chronic stroke patients when combined with physiotherapy (Allman et al., 2016). However, the effectiveness of anodal tDCS in the subacute stage after stroke has not been thoroughly investigated, despite the fact that this is the time when the majority of rehabilitation takes place. Although anodal and cathodal arrangements led to similar improvements in the current study, Stinear et al. (2015) speculate that directly facilitating ipsilesional M1 excitability is likely to better promote recovery at the subacute stage of stroke than suppressing contralesional M1 excitability. Their study tracked patients for 26 weeks following stroke and found that increases in ipsilesional M1 excitability were associated with reductions in impairment and improvements in function. There was no evidence for a reduction in contralesional M1 hyperexcitability during recovery, nor was there an imbalance in TCI throughout the 26 week period.

7.6 Future directions

Since the experiments for this thesis were designed and implemented the number of studies examining the effect of tDCS on function after stroke has continued to increase. The findings of this thesis, together with these recent publications, lead to a number of suggestions for aims for future studies:

- To test for any specific role of the left M1 in motor sequence learning.
- To further investigate the possibility that cathodal tDCS delivered in a training session could impair subsequent learning on the same task.
- To determine whether people with the dominant hand affected by stroke show greater improvements in function after repeated sessions of tDCS than those with the non-dominant affected.
- To further investigate the role of stroke location in the response to tDCS.
- To assess whether stroke survivors also show impairment in the re-learning of functional upper limb movements that are required for activities of daily living.
- To investigate the role of changes in functional connectivity and GABA concentration in the improvement of function with repeated sessions of tDCS.
- To investigate the effect of anodal tDCS on the rate and amount of recovery over the first six months after stroke.

7.7 Conclusion

Although the influence of tDCS electrode arrangement on motor sequence learning remains unclear, only unilateral tDCS shows efficacy in improving upper limb function after stroke. These findings have implications for the design of future clinical trials to assess whether tDCS is a viable strategy to enhance physical therapy and improve functional independence in stroke survivors.

References

Ackerley, S.J., Stinear, C.M., Barber, P.A., Byblow, W.D., 2010. Combining theta burst stimulation with training after subcortical stroke. Stroke. 41**,** 1568-72.

Allman, C., Amadi, U., Winkler, A.M., Wilkins, L., Filippini, N., Kischka, U., Stagg, C.J., Johansen-Berg, H., 2016. Ipsilesional anodal tDCS enhances the functional benefits of rehabilitation in patients after stroke. Sci Transl Med. 8**,** 330re1.

Amadi, U., Allman, C., Johansen-Berg, H., Stagg, C.J., 2015. The Homeostatic Interaction Between Anodal Transcranial Direct Current Stimulation and Motor Learning in Humans is Related to GABAA Activity. Brain Stimul. 8**,** 898-905.

Ambrus, G.G., Chaieb, L., Stilling, R., Rothkegel, H., Antal, A., Paulus, W., 2016. Monitoring transcranial direct current stimulation induced changes in cortical excitability during the serial reaction time task. Neurosci Lett. 616**,** 98-104.

Ardolino, G., Bossi, B., Barbieri, S., Priori, A., 2005. Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. J Physiol. 568**,** 653-63.

Au-Yeung, S.S., Wang, J., Chen, Y., Chua, E., 2014. Transcranial direct current stimulation to primary motor area improves hand dexterity and selective attention in chronic stroke. Am J Phys Med Rehabil. 93**,** 1057-64.

Bastani, A., Jaberzadeh, S., 2012. Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: a systematic review and meta-analysis. Clin Neurophysiol. 123**,** 644-57.

Bastani, A., Jaberzadeh, S., 2013a. Differential modulation of corticospinal excitability by different current densities of anodal transcranial direct current stimulation. PLoS One. 8**,** e72254.

Bastani, A., Jaberzadeh, S., 2013b. a-tDCS differential modulation of corticospinal excitability: the effects of electrode size. Brain Stimul. 6**,** 932-7.

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M.F., Nitsche, M.A., 2013. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol. 591**,** 1987-2000.

Blicher, J.U., Near, J., Naess-Schmidt, E., Stagg, C.J., Johansen-Berg, H., Nielsen, J.F., Ostergaard, L., Ho, Y.C., 2015. GABA levels are decreased after stroke and GABA changes during rehabilitation correlate with motor improvement. Neurorehabil Neural Repair. 29**,** 278-86.

Boggio, P.S., Nunes, A., Rigonatti, S.P., Nitsche, M.A., Pascual-Leone, A., Fregni, F., 2007. Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. Restor Neurol Neurosci. 25**,** 123-9.

Bolognini, N., Vallar, G., Casati, C., Latif, L.A., El-Nazer, R., Williams, J., Banco, E., Macea, D.D., Tesio, L., Chessa, C., Fregni, F., 2011. Neurophysiological and behavioral effects of tDCS combined with constraint-induced movement therapy in poststroke patients. Neurorehabil Neural Repair. 25**,** 819-29.

Boyd, L.A., Winstein, C.J., 2001. Implicit motor-sequence learning in humans following unilateral stroke: the impact of practice and explicit knowledge. Neurosci Lett. 298**,** 65-9.

Boyd, L.A., Winstein, C.J., 2003. Impact of explicit information on implicit motor-sequence learning following middle cerebral artery stroke. Phys Ther. 83**,** 976-89.

Boyd, L.A., Quaney, B.M., Pohl, P.S., Winstein, C.J., 2007. Learning implicitly: effects of task and severity after stroke. Neurorehabil Neural Repair. 21**,** 444-54.

Boyd, L.A., Vidoni, E.D., Siengsukon, C.F., 2008. Multidimensional motor sequence learning is impaired in older but not younger or middle-aged adults. Phys Ther. 88**,** 351-62.

Bradnam, L.V., Stinear, C.M., Barber, P.A., Byblow, W.D., 2012. Contralesional hemisphere control of the proximal paretic upper limb following stroke. Cereb Cortex. 22**,** 2662-71.

Bradnam, L.V., Stinear, C.M., Byblow, W.D., 2013. Ipsilateral motor pathways after stroke: implications for non-invasive brain stimulation. Front Hum Neurosci. 7**,** 184.

Buetefisch, C.M., 2015. Role of the Contralesional Hemisphere in Post-Stroke Recovery of Upper Extremity Motor Function. Front Neurol. 6**,** 214.

Butler, A.J., Shuster, M., O'Hara, E., Hurley, K., Middlebrooks, D., Guilkey, K., 2013. A meta-analysis of the efficacy of anodal transcranial direct current stimulation for upper limb motor recovery in stroke survivors. J Hand Ther. 26**,** 162-70; quiz 171.

Celnik, P., Paik, N.J., Vandermeeren, Y., Dimyan, M., Cohen, L.G., 2009. Effects of combined peripheral nerve stimulation and brain polarization on performance of a motor sequence task after chronic stroke. Stroke. 40**,** 1764-71.

Censor, N., Sagi, D., Cohen, L.G., 2012. Common mechanisms of human perceptual and motor learning. Nat Rev Neurosci. 13**,** 658-64.

Chen, R., Yung, D., Li, J.Y., 2003. Organization of ipsilateral excitatory and inhibitory pathways in the human motor cortex. J Neurophysiol. 89**,** 1256-64.

Chhatbar, P.Y., Ramakrishnan, V., Kautz, S., George, M.S., Adams, R.J., Feng, W., 2016. Transcranial Direct Current Stimulation Post-Stroke Upper Extremity Motor Recovery Studies Exhibit a Dose-Response Relationship. Brain Stimul. 9**,** 16-26.

Coppi, E., Houdayer, E., Chieffo, R., Spagnolo, F., Inuggi, A., Straffi, L., Comi, G., Leocani, L., 2014. Age-related changes in motor cortical representation and interhemispheric interactions: a transcranial magnetic stimulation study. Front Aging Neurosci. 6**,** 209.

Cunningham, D.A., Machado, A., Janini, D., Varnerin, N., Bonnett, C., Yue, G., Jones, S., Lowe, M., Beall, E., Sakaie, K., Plow, E.B., 2015. Assessment of inter-hemispheric imbalance using imaging and noninvasive brain stimulation in patients with chronic stroke. Arch Phys Med Rehabil. 96**,** S94-103.

Curran, T., 1997. Effects of aging on implicit sequence learning: accounting for sequence structure and explicit knowledge. Psychol Res. 60**,** 24-41.

Davidson, T., Tremblay, F., 2013. Age and hemispheric differences in transcallosal inhibition between motor cortices: an ispsilateral silent period study. BMC Neurosci. 14**,** 62.

Davidson, T.W., Bolic, M., Tremblay, F., 2016. Predicting Modulation in Corticomotor Excitability and in Transcallosal Inhibition in Response to Anodal Transcranial Direct Current Stimulation. Front Hum Neurosci. 10**,** 49.

Dayan, E., Cohen, L.G., 2011. Neuroplasticity subserving motor skill learning. Neuron. 72**,** 443-54.

DeCarli, C., Kawas, C., Morrison, J.H., Reuter-Lorenz, P.A., Sperling, R.A., Wright, C.B., 2012. Session II: Mechanisms of age-related cognitive change and targets for intervention: neural circuits, networks, and plasticity. J Gerontol A Biol Sci Med Sci. 67**,** 747-53.

Der, G., Deary, I.J., 2006. Age and sex differences in reaction time in adulthood: results from the United Kingdom Health and Lifestyle Survey. Psychol Aging. 21**,** 62-73.

Di Lazzaro, V., Manganelli, F., Dileone, M., Notturno, F., Esposito, M., Capasso, M., Dubbioso, R., Pace, M., Ranieri, F., Minicuci, G., Santoro, L., Uncini, A., 2012. The effects of prolonged cathodal direct current stimulation on the excitatory and inhibitory circuits of the ipsilateral and contralateral motor cortex. J Neural Transm (Vienna). 119**,** 1499-506.

Di Lazzaro, V., Dileone, M., Capone, F., Pellegrino, G., Ranieri, F., Musumeci, G., Florio, L., Di Pino, G., Fregni, F., 2014. Immediate and late modulation of interhemipheric imbalance with bilateral transcranial direct current stimulation in acute stroke. Brain Stimul. 7**,** 841-8.

Dobkin, B.H., 2005. Clinical practice. Rehabilitation after stroke. N Engl J Med. 352**,** 1677-84.

Doherty, T.J., 2003. Invited review: Aging and sarcopenia. J Appl Physiol (1985). 95**,** 1717-27.

Dovern, A., Fink, G.R., Saliger, J., Karbe, H., Koch, I., Weiss, P.H., 2011. Apraxia impairs intentional retrieval of incidentally acquired motor knowledge. J Neurosci. 31**,** 8102-8.

Dyke, K., Kim, S., Jackson, G.M., Jackson, S.R., 2016. Intra-Subject Consistency and Reliability of Response Following 2 mA Transcranial Direct Current Stimulation. Brain Stimul.

Exner, C., Koschack, J., Irle, E., 2002. The differential role of premotor frontal cortex and basal ganglia in motor sequence learning: evidence from focal basal ganglia lesions. Learn Mem. 9**,** 376-86.

Fadiga, L., Buccino, G., Craighero, L., Fogassi, L., Gallese, V., Pavesi, G., 1999. Corticospinal excitability is specifically modulated by motor imagery: a magnetic stimulation study. Neuropsychologia. 37**,** 147-58.

Fleming, M.K., Sorinola, I.O., Newham, D.J., Roberts-Lewis, S.F., Bergmann, J.H., 2012. The effect of coil type and navigation on the reliability of transcranial magnetic stimulation. IEEE Trans Neural Syst Rehabil Eng. 20**,** 617-25.

Francis, K.L., Spirduso, W.W., 2000. Age differences in the expression of manual asymmetry. Exp Aging Res. 26**,** 169-80.

Fregni, F., Boggio, P.S., Mansur, C.G., Wagner, T., Ferreira, M.J., Lima, M.C., Rigonatti, S.P., Marcolin, M.A., Freedman, S.D., Nitsche, M.A., Pascual-Leone, A., 2005. Transcranial direct current stimulation of the unaffected hemisphere in stroke patients. Neuroreport. 16**,** 1551-5.

Fricke, K., Seeber, A.A., Thirugnanasambandam, N., Paulus, W., Nitsche, M.A., Rothwell, J.C., 2011. Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol. 105**,** 1141-9.

Fugl-Meyer, A.R., Jaasko, L., Leyman, I., Olsson, S., Steglind, S., 1975. The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. Scand J Rehabil Med. 7**,** 13-31.

Fujiyama, H., Hyde, J., Hinder, M.R., Kim, S.J., McCormack, G.H., Vickers, J.C., Summers, J.J., 2014. Delayed plastic responses to anodal tDCS in older adults. Front Aging Neurosci. 6**,** 115.

Fusco, A., De Angelis, D., Morone, G., Maglione, L., Paolucci, T., Bragoni, M., Venturiero, V., 2013. The ABC of tDCS: Effects of Anodal, Bilateral and Cathodal Montages of Transcranial Direct Current Stimulation in Patients with Stroke-A Pilot Study. Stroke Res Treat. 2013**,** 837595.

Fusco, A., Assenza, F., Iosa, M., Izzo, S., Altavilla, R., Paolucci, S., Vernieri, F., 2014a. The ineffective role of cathodal tDCS in enhancing the functional motor outcomes in early phase of stroke rehabilitation: an experimental trial. Biomed Res Int. 2014**,** 547290.

Fusco, A., Iosa, M., Venturiero, V., De Angelis, D., Morone, G., Maglione, L., Bragoni, M., Coiro, P., Pratesi, L., Paolucci, S., 2014b. After vs. priming effects of anodal transcranial direct current stimulation on upper extremity motor recovery in patients with subacute stroke. Restor Neurol Neurosci. 32**,** 301-12.

Giovannelli, F., Borgheresi, A., Balestrieri, F., Zaccara, G., Viggiano, M.P., Cincotta, M., Ziemann, U., 2009. Modulation of interhemispheric inhibition by volitional motor activity: an ipsilateral silent period study. J Physiol. 587**,** 5393-410.

Goh, H.T., Chan, H.Y., Abdul-Latif, L., 2015. Aftereffects of 2 noninvasive brain stimulation techniques on corticospinal excitability in persons with chronic stroke: a pilot study. J Neurol Phys Ther. 39**,** 15-22.

Gomes-Osman, J., Field-Fote, E.C., 2013. Bihemispheric anodal corticomotor stimulation using transcranial direct current stimulation improves bimanual typing task performance. J Mot Behav. 45**,** 361-7.

Goodwill, A.M., Reynolds, J., Daly, R.M., Kidgell, D.J., 2013. Formation of cortical plasticity in older adults following tDCS and motor training. Front Aging Neurosci. 5**,** 87.

Gryga, M., Taubert, M., Dukart, J., Vollmann, H., Conde, V., Sehm, B., Villringer, A., Ragert, P., 2012. Bidirectional gray matter changes after complex motor skill learning. Front Syst Neurosci. 6**,** 37.

Hardwick, R.M., Rottschy, C., Miall, R.C., Eickhoff, S.B., 2013. A quantitative meta-analysis and review of motor learning in the human brain. Neuroimage. 67**,** 283-97.

Hashemirad, F., Zoghi, M., Fitzgerald, P.B., Jaberzadeh, S., 2016. The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals: A systematic review and meta-analysis. Brain Cogn. 102**,** 1-12.

Hesse, S., Waldner, A., Mehrholz, J., Tomelleri, C., Pohl, M., Werner, C., 2011. Combined transcranial direct current stimulation and robot-assisted arm training in subacute stroke patients: an exploratory, randomized multicenter trial. Neurorehabil Neural Repair. 25**,** 838-46.

Hikosaka, O., Nakamura, K., Sakai, K., Nakahara, H., 2002. Central mechanisms of motor skill learning. Curr Opin Neurobiol. 12**,** 217-22.

Horvath, J.C., Vogrin, S.J., Carter, O., Cook, M.J., Forte, J.D., 2016. Effects of a common transcranial direct current stimulation (tDCS) protocol on motor evoked potentials found to be highly variable within individuals over 9 testing sessions. Exp Brain Res. 234**,** 2629-42.

Hoshi, E., Tanji, J., 2007. Distinctions between dorsal and ventral premotor areas: anatomical connectivity and functional properties. Curr Opin Neurobiol. 17**,** 234-42.

Howard, D.V., Howard, J.H., Jr., Japikse, K., DiYanni, C., Thompson, A., Somberg, R., 2004. Implicit sequence learning: effects of level of structure, adult age, and extended practice. Psychol Aging. 19**,** 79-92.

Hoyer, E.H., Celnik, P.A., 2011. Understanding and enhancing motor recovery after stroke using transcranial magnetic stimulation. Restor Neurol Neurosci. 29**,** 395-409.

Huang, V.S., Haith, A., Mazzoni, P., Krakauer, J.W., 2011. Rethinking motor learning and savings in adaptation paradigms: model-free memory for successful actions combines with internal models. Neuron. 70**,** 787-801.

Hubbard, I.J., Parsons, M.W., Neilson, C., Carey, L.M., 2009. Task-specific training: evidence for and translation to clinical practice. Occup Ther Int. 16**,** 175-89.

Hummel, F., Celnik, P., Giraux, P., Floel, A., Wu, W.H., Gerloff, C., Cohen, L.G., 2005. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. Brain. 128**,** 490-9.

Hummel, F.C., Voller, B., Celnik, P., Floel, A., Giraux, P., Gerloff, C., Cohen, L.G., 2006. Effects of brain polarization on reaction times and pinch force in chronic stroke. BMC Neurosci. 7**,** 73.

Hummel, F.C., Heise, K., Celnik, P., Floel, A., Gerloff, C., Cohen, L.G., 2010. Facilitating skilled right hand motor function in older subjects by anodal polarization over the left primary motor cortex. Neurobiol Aging. 31**,** 2160-8.

Jebsen, R.H., Taylor, N., Trieschmann, R.B., Trotter, M.J., Howard, L.A., 1969. An objective and standardized test of hand function. Arch Phys Med Rehabil. 50**,** 311-9.

Kang, E.K., Paik, N.J., 2011. Effect of a tDCS electrode montage on implicit motor sequence learning in healthy subjects. Exp Transl Stroke Med. 3**,** 4.

Kang, N., Summers, J.J., Cauraugh, J.H., 2016. Transcranial direct current stimulation facilitates motor learning post-stroke: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 87**,** 345-55.

Kantak, S.S., Mummidisetty, C.K., Stinear, J.W., 2012. Primary motor and premotor cortex in implicit sequence learning--evidence for competition between implicit and explicit human motor memory systems. Eur J Neurosci. 36**,** 2710-5.

Karni, A., Meyer, G., Rey-Hipolito, C., Jezzard, P., Adams, M.M., Turner, R., Ungerleider, L.G., 1998. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proc Natl Acad Sci U S A. 95**,** 861-8.

Karok, S., Witney, A.G., 2013. Enhanced motor learning following task-concurrent dual transcranial direct current stimulation. PLoS One. 8**,** e85693.

Khedr, E.M., Shawky, O.A., El-Hammady, D.H., Rothwell, J.C., Darwish, E.S., Mostafa, O.M., Tohamy, A.M., 2013. Effect of anodal versus cathodal transcranial direct current stimulation on stroke rehabilitation: a pilot randomized controlled trial. Neurorehabil Neural Repair. 27**,** 592-601.

Kidgell, D.J., Daly, R.M., Young, K., Lum, J., Tooley, G., Jaberzadeh, S., Zoghi, M., Pearce, A.J., 2013a. Different current intensities of anodal transcranial direct current stimulation do not differentially modulate motor cortex plasticity. Neural Plast. 2013**,** 603502.

Kidgell, D.J., Goodwill, A.M., Frazer, A.K., Daly, R.M., 2013b. Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex. BMC Neurosci. 14**,** 64.

Kim, D.Y., Ohn, S.H., Yang, E.J., Park, C.I., Jung, K.J., 2009. Enhancing motor performance by anodal transcranial direct current stimulation in subacute stroke patients. Am J Phys Med Rehabil. 88**,** 829-36.

Kim, D.Y., Lim, J.Y., Kang, E.K., You, D.S., Oh, M.K., Oh, B.M., Paik, N.J., 2010. Effect of transcranial direct current stimulation on motor recovery in patients with subacute stroke. Am J Phys Med Rehabil. 89**,** 879-86.

Kim, G.W., Ko, M.H., 2013. Facilitation of corticospinal tract excitability by transcranial direct current stimulation combined with voluntary grip exercise. Neurosci Lett. 548**,** 181-4.

Kim, S., Stephenson, M.C., Morris, P.G., Jackson, S.R., 2014. tDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: a 7 T magnetic resonance spectroscopy study. Neuroimage. 99**,** 237-43.

Kim, S.G., Ashe, J., Hendrich, K., Ellermann, J.M., Merkle, H., Ugurbil, K., Georgopoulos, A.P., 1993. Functional magnetic resonance imaging of motor cortex: hemispheric asymmetry and handedness. Science. 261**,** 615-7.

Kitago, T., Ryan, S.L., Mazzoni, P., Krakauer, J.W., Haith, A.M., 2013. Unlearning versus savings in visuomotor adaptation: comparing effects of washout, passage of time, and removal of errors on motor memory. Front Hum Neurosci. 7**,** 307.

Kleim, J.A., Barbay, S., Nudo, R.J., 1998. Functional reorganization of the rat motor cortex following motor skill learning. J Neurophysiol. 80**,** 3321-5.

Krakauer, J.W., 2006. Motor learning: its relevance to stroke recovery and neurorehabilitation. Curr Opin Neurol. 19**,** 84-90.

Lang, N., Nitsche, M.A., Paulus, W., Rothwell, J.C., Lemon, R.N., 2004. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Exp Brain Res. 156**,** 439-43.

Lefebvre, S., Dricot, L., Gradkowski, W., Laloux, P., Vandermeeren, Y., 2012a. Brain activations underlying different patterns of performance improvement during early motor skill learning. Neuroimage. 62**,** 290-9.

Lefebvre, S., Laloux, P., Peeters, A., Desfontaines, P., Jamart, J., Vandermeeren, Y., 2012b. Dual-tDCS Enhances Online Motor Skill Learning and Long-Term Retention in Chronic Stroke Patients. Front Hum Neurosci. 6**,** 343.

Lefebvre, S., Thonnard, J.L., Laloux, P., Peeters, A., Jamart, J., Vandermeeren, Y., 2014. Single session of dual-tDCS transiently improves precision grip and dexterity of the paretic hand after stroke. Neurorehabil Neural Repair. 28**,** 100-10.

Lefebvre, S., Dricot, L., Laloux, P., Gradkowski, W., Desfontaines, P., Evrard, F., Peeters, A., Jamart, J., Vandermeeren, Y., 2015. Neural substrates underlying stimulation-enhanced motor skill learning after stroke. Brain. 138**,** 149-63.

Leow, L.A., Hammond, G., de Rugy, A., 2014. Anodal motor cortex stimulation paired with movement repetition increases anterograde interference but not savings. Eur J Neurosci. 40**,** 3243-52.

Li, L.M., Uehara, K., Hanakawa, T., 2015. The contribution of interindividual factors to variability of response in transcranial direct current stimulation studies. Front Cell Neurosci. 9**,** 181.

Liebetanz, D., Nitsche, M.A., Tergau, F., Paulus, W., 2002. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain. 125**,** 2238-47.

Lindenberg, R., Renga, V., Zhu, L.L., Nair, D., Schlaug, G., 2010. Bihemispheric brain stimulation facilitates motor recovery in chronic stroke patients. Neurology. 75**,** 2176-84.

Lindenberg, R., Nachtigall, L., Meinzer, M., Sieg, M.M., Floel, A., 2013. Differential effects of dual and unihemispheric motor cortex stimulation in older adults. J Neurosci. 33**,** 9176-83.

Lindenberg, R., Sieg, M.M., Meinzer, M., Nachtigall, L., Floel, A., 2016. Neural correlates of unihemispheric and bihemispheric motor cortex stimulation in healthy young adults. Neuroimage.

Lopez-Alonso, V., Cheeran, B., Rio-Rodriguez, D., Fernandez-Del-Olmo, M., 2014. Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul. 7**,** 372-80.

Lotze, M., Markert, J., Sauseng, P., Hoppe, J., Plewnia, C., Gerloff, C., 2006. The role of multiple contralesional motor areas for complex hand movements after internal capsular lesion. J Neurosci. 26**,** 6096-102.

Ludemann-Podubecka, J., Bosl, K., Theilig, S., Wiederer, R., Nowak, D.A., 2015. The Effectiveness of 1 Hz rTMS Over the Primary Motor Area of the Unaffected Hemisphere to Improve Hand Function After Stroke Depends on Hemispheric Dominance. Brain Stimul. 8**,** 823-30.

Mahmoudi, H., Borhani Haghighi, A., Petramfar, P., Jahanshahi, S., Salehi, Z., Fregni, F., 2011. Transcranial direct current stimulation: electrode montage in stroke. Disabil Rehabil. 33**,** 1383-8.

Marquez, J., van Vliet, P., McElduff, P., Lagopoulos, J., Parsons, M., 2015. Transcranial direct current stimulation (tDCS): does it have merit in stroke rehabilitation? A systematic review. Int J Stroke. 10**,** 306-16.

Matsuo, A., Maeoka, H., Hiyamizu, M., Shomoto, K., Morioka, S., Seki, K., 2011. Enhancement of precise hand movement by transcranial direct current stimulation. Neuroreport. 22**,** 78-82.

Medeiros, L.F., de Souza, I.C., Vidor, L.P., de Souza, A., Deitos, A., Volz, M.S., Fregni, F., Caumo, W., Torres, I.L., 2012. Neurobiological effects of transcranial direct current stimulation: a review. Front Psychiatry. 3**,** 110.

Moisello, C., Crupi, D., Tunik, E., Quartarone, A., Bove, M., Tononi, G., Ghilardi, M.F., 2009. The serial reaction time task revisited: a study on motor sequence learning with an arm-reaching task. Exp Brain Res. 194**,** 143-55.

Moliadze, V., Antal, A., Paulus, W., 2010. Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. Clin Neurophysiol. 121**,** 2165-71.

Moliadze, V., Fritzsche, G., Antal, A., 2014. Comparing the efficacy of excitatory transcranial stimulation methods measuring motor evoked potentials. Neural Plast. 2014**,** 837141.

Monte-Silva, K., Kuo, M.F., Liebetanz, D., Paulus, W., Nitsche, M.A., 2010. Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). J Neurophysiol. 103**,** 1735-40.

Monte-Silva, K., Kuo, M.F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., Nitsche, M.A., 2013. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul. 6**,** 424-32.

Mortensen, J., Figlewski, K., Andersen, H., 2016. Combined transcranial direct current stimulation and home-based occupational therapy for upper limb motor impairment following intracerebral hemorrhage: a double-blind randomized controlled trial. Disabil Rehabil. 38**,** 637-43.

Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S., Boroojerdi, B., Poewe, W., Hallett, M., 2002. Early consolidation in human primary motor cortex. Nature. 415**,** 640-4.

Murase, N., Duque, J., Mazzocchio, R., Cohen, L.G., 2004. Influence of interhemispheric interactions on motor function in chronic stroke. Ann Neurol. 55**,** 400-9.

Nair, D.G., Purcott, K.L., Fuchs, A., Steinberg, F., Kelso, J.A., 2003. Cortical and cerebellar activity of the human brain during imagined and executed unimanual and bimanual action sequences: a functional MRI study. Brain Res Cogn Brain Res. 15**,** 250-60.

Naros, G., Geyer, M., Koch, S., Mayr, L., Ellinger, T., Grimm, F., Gharabaghi, A., 2016. Enhanced motor learning with bilateral transcranial direct current stimulation: Impact of polarity or current flow direction? Clin Neurophysiol. 127**,** 2119-26.

Nissen, M.J., Bullemer, P., 1987. Attentional requirements of learning: Evidence from performance measures. Cognitive Psychology. 19**,** 1-32.

Nitsche, M.A., Paulus, W., 2000. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 527 Pt 3**,** 633-9.

Nitsche, M.A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., Henning, S., Tergau, F., Paulus, W., 2003a. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol. 553**,** 293-301.

Nitsche, M.A., Nitsche, M.S., Klein, C.C., Tergau, F., Rothwell, J.C., Paulus, W., 2003b. Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin Neurophysiol. 114**,** 600-4.

Nitsche, M.A., Schauenburg, A., Lang, N., Liebetanz, D., Exner, C., Paulus, W., Tergau, F., 2003c. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. J Cogn Neurosci. 15**,** 619-26.

Nitsche, M.A., Seeber, A., Frommann, K., Klein, C.C., Rochford, C., Nitsche, M.S., Fricke, K., Liebetanz, D., Lang, N., Antal, A., Paulus, W., Tergau, F., 2005. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol. 568**,** 291-303.

Nowak, D.A., Grefkes, C., Ameli, M., Fink, G.R., 2009. Interhemispheric competition after stroke: brain stimulation to enhance recovery of function of the affected hand. Neurorehabil Neural Repair. 23**,** 641-56.

O'Shea, J., Boudrias, M.H., Stagg, C.J., Bachtiar, V., Kischka, U., Blicher, J.U., Johansen-Berg, H., 2014. Predicting behavioural response to TDCS in chronic motor stroke. Neuroimage. 85 Pt 3**,** 924-33.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 9**,** 97-113.

Opitz, A., Paulus, W., Will, S., Antunes, A., Thielscher, A., 2015. Determinants of the electric field during transcranial direct current stimulation. Neuroimage. 109**,** 140-50.

Orrell, A.J., Eves, F.F., Masters, R.S., MacMahon, K.M., 2007. Implicit sequence learning processes after unilateral stroke. Neuropsychol Rehabil. 17**,** 335-54.

Parikh, P.J., Cole, K.J., 2014. Effects of transcranial direct current stimulation in combination with motor practice on dexterous grasping and manipulation in healthy older adults. Physiol Rep. 2**,** e00255.

Parikh, P.J., Cole, K.J., 2015. Effects of transcranial direct current stimulation on the control of finger force during dexterous manipulation in healthy older adults. PLoS One. 10**,** e0124137.

Penhune, V.B., Steele, C.J., 2012. Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. Behav Brain Res. 226**,** 579-91.

Perruchet, P., Bigand, E., Benoit-Gonin, F., 1997. The emergence of explicit knowledge during the early phase of learning in sequential reaction time tasks. Psychol Res. 60**,** 4-13.

Pohl, P.S., McDowd, J.M., Filion, D.L., Richards, L.G., Stiers, W., 2001. Implicit learning of a perceptual-motor skill after stroke. Phys Ther. 81**,** 1780-9.

Pohl, P.S., McDowd, J.M., Filion, D., Richards, L.G., Stiers, W., 2006. Implicit learning of a motor skill after mild and moderate stroke. Clin Rehabil. 20**,** 246-53.

Prichard, G., Weiller, C., Fritsch, B., Reis, J., 2014. Effects of different electrical brain stimulation protocols on subcomponents of motor skill learning. Brain Stimul. 7**,** 532-40.

Reis, J., Robertson, E., Krakauer, J.W., Rothwell, J., Marshall, L., Gerloff, C., Wassermann, E., Pascual-Leone, A., Hummel, F., Celnik, P.A., Classen, J., Floel, A., Ziemann, U., Paulus, W., Siebner, H.R., Born, J., Cohen, L.G., 2008. Consensus: "Can tDCS and TMS enhance motor learning and memory formation?". Brain Stimul. 1**,** 363-369.

Reis, J., Schambra, H.M., Cohen, L.G., Buch, E.R., Fritsch, B., Zarahn, E., Celnik, P.A., Krakauer, J.W., 2009. Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc Natl Acad Sci U S A. 106**,** 1590-5.

Reuter-Lorenz, P.A., Lustig, C., 2005. Brain aging: reorganizing discoveries about the aging mind. Curr Opin Neurobiol. 15**,** 245-51.

Richardson, A.G., Overduin, S.A., Valero-Cabre, A., Padoa-Schioppa, C., Pascual-Leone, A., Bizzi, E., Press, D.Z., 2006. Disruption of primary motor cortex before learning impairs memory of movement dynamics. J Neurosci. 26**,** 12466-70.

Rioult-Pedotti, M.S., Friedman, D., Donoghue, J.P., 2000. Learning-induced LTP in neocortex. Science. 290**,** 533-6.

Robertson, E.M., Pascual-Leone, A., Miall, R.C., 2004. Current concepts in procedural consolidation. Nat Rev Neurosci. 5**,** 576-82.

Rocha, S., Silva, E., Foerster, A., Wiesiolek, C., Chagas, A.P., Machado, G., Baltar, A., Monte-Silva, K., 2016. The impact of transcranial direct current stimulation (tDCS) combined with modified constraint-induced movement therapy (mCIMT) on upper limb function in chronic stroke: a double-blind randomized controlled trial. Disabil Rehabil. 38**,** 653-60.

Roche, N., Geiger, M., Bussel, B., 2015. Mechanisms underlying transcranial direct current stimulation in rehabilitation. Ann Phys Rehabil Med. 58**,** 214-9.

Rom, D., 1990. A sequentially rejective test procedure based on a modified Bonferroni inequality. Biometrika. 77**,** 663-665.

Rroji, O., van Kuyck, K., Nuttin, B., Wenderoth, N., 2015. Anodal tDCS over the Primary Motor Cortex Facilitates Long-Term Memory Formation Reflecting Use-Dependent Plasticity. PLoS One. 10**,** e0127270.

Sampaio-Baptista, C., Scholz, J., Jenkinson, M., Thomas, A.G., Filippini, N., Smit, G., Douaud, G., Johansen-Berg, H., 2014. Gray matter volume is associated with rate of subsequent skill learning after a long term training intervention. Neuroimage. 96**,** 158-66.

Sarmiento, C.I., San-Juan, D., Prasath, V.B., 2016. Letter to the Editor: Brief history of transcranial direct current stimulation (tDCS): from electric fishes to microcontrollers. Psychol Med. 1-3.

Sattler, V., Acket, B., Raposo, N., Albucher, J.F., Thalamas, C., Loubinoux, I., Chollet, F., Simonetta-Moreau, M., 2015. Anodal tDCS Combined With Radial Nerve Stimulation Promotes Hand Motor Recovery in the Acute Phase After Ischemic Stroke. Neurorehabil Neural Repair. 29**,** 743-54.

Savic, B., Meier, B., 2016. How Transcranial Direct Current Stimulation Can Modulate Implicit Motor Sequence Learning and Consolidation: A Brief Review. Front Hum Neurosci. 10**,** 26.

Schade, S., Moliadze, V., Paulus, W., Antal, A., 2012. Modulating neuronal excitability in the motor cortex with tDCS shows moderate hemispheric asymmetry due to subjects' handedness: a pilot study. Restor Neurol Neurosci. 30**,** 191-8.

Sehm, B., Schafer, A., Kipping, J., Margulies, D., Conde, V., Taubert, M., Villringer, A., Ragert, P., 2012. Dynamic modulation of intrinsic functional connectivity by transcranial direct current stimulation. J Neurophysiol. 108**,** 3253-63.

Sehm, B., Kipping, J., Schafer, A., Villringer, A., Ragert, P., 2013. A Comparison between Uni- and Bilateral tDCS Effects on Functional Connectivity of the Human Motor Cortex. Front Hum Neurosci. 7**,** 183.

Seidler-Dobrin, R.D., He, J., Stelmach, G.E., 1998. Coactivation to reduce variability in the elderly. Motor Control. 2**,** 314-30.

Seidler, R., Erdeniz, B., Koppelmans, V., Hirsiger, S., Merillat, S., Jancke, L., 2015. Associations between age, motor function, and resting state sensorimotor network connectivity in healthy older adults. Neuroimage. 108**,** 47-59.

Seidler, R.D., Bernard, J.A., Burutolu, T.B., Fling, B.W., Gordon, M.T., Gwin, J.T., Kwak, Y., Lipps, D.B., 2010. Motor control and aging: links to age-related brain structural, functional, and biochemical effects. Neurosci Biobehav Rev. 34**,** 721-33.

Shmuelof, L., Krakauer, J.W., 2011. Are we ready for a natural history of motor learning? Neuron. 72**,** 469-76.

Stagg, C.J., Best, J.G., Stephenson, M.C., O'Shea, J., Wylezinska, M., Kincses, Z.T., Morris, P.G., Matthews, P.M., Johansen-Berg, H., 2009. Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. J Neurosci. 29**,** 5202-6.

Stagg, C.J., Jayaram, G., Pastor, D., Kincses, Z.T., Matthews, P.M., Johansen-Berg, H., 2011. Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning. Neuropsychologia. 49**,** 800-4.

Stagg, C.J., Nitsche, M.A., 2011. Physiological basis of transcranial direct current stimulation. Neuroscientist. 17**,** 37-53.

Stagg, C.J., Johansen-Berg, H., 2013. Studying the effects of transcranial direct-current stimulation in stroke recovery using magnetic resonance imaging. Front Hum Neurosci. 7**,** 857.

Stefan, K., Wycislo, M., Gentner, R., Schramm, A., Naumann, M., Reiners, K., Classen, J., 2006. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb Cortex. 16**,** 376-85.

Stinear, C.M., Fleming, M.K., Byblow, W.D., 2006. Lateralization of unimanual and bimanual motor imagery. Brain Res. 1095**,** 139-47.

Stinear, C.M., Barber, P.A., Smale, P.R., Coxon, J.P., Fleming, M.K., Byblow, W.D., 2007. Functional potential in chronic stroke patients depends on corticospinal tract integrity. Brain. 130**,** 170-80.

Stinear, C.M., Barber, P.A., Petoe, M., Anwar, S., Byblow, W.D., 2012. The PREP algorithm predicts potential for upper limb recovery after stroke. Brain. 135**,** 2527-35.

Stinear, C.M., Petoe, M.A., Byblow, W.D., 2015. Primary Motor Cortex Excitability During Recovery After Stroke: Implications for Neuromodulation. Brain Stimul. 8**,** 1183-90.

Strube, W., Bunse, T., Nitsche, M.A., Nikolaeva, A., Palm, U., Padberg, F., Falkai, P., Hasan, A., 2016. Bidirectional variability in motor cortex excitability modulation following 1 mA transcranial direct current stimulation in healthy participants. Physiol Rep. 4.

Takeuchi, N., Tada, T., Toshima, M., Ikoma, K., 2010. Correlation of motor function with transcallosal and intracortical inhibition after stroke. J Rehabil Med. 42**,** 962-6.

Takeuchi, N., Izumi, S., 2012. Maladaptive plasticity for motor recovery after stroke: mechanisms and approaches. Neural Plast. 2012**,** 359728.

Tazoe, T., Endoh, T., Kitamura, T., Ogata, T., 2014. Polarity specific effects of transcranial direct current stimulation on interhemispheric inhibition. PLoS One. 9**,** e114244.

Tremblay, S., Beaule, V., Lepage, J.F., Theoret, H., 2013. Anodal transcranial direct current stimulation modulates GABAB-related intracortical inhibition in the M1 of healthy individuals. Neuroreport. 24**,** 46-50.

Tremblay, S., Lafleur, L.P., Proulx, S., Beaule, V., Latulipe-Loiselle, A., Doyon, J., Marjanska, M., Theoret, H., 2016. The effects of bi-hemispheric M1-M1 transcranial direct current stimulation on primary motor cortex neurophysiology and metabolite concentration. Restor Neurol Neurosci. 34**,** 587-602.

Vecchio, F., Miraglia, F., Bramanti, P., Rossini, P.M., 2014. Human brain networks in physiological aging: a graph theoretical analysis of cortical connectivity from EEG data. J Alzheimers Dis. 41**,** 1239-49.

Veerbeek, J.M., Kwakkel, G., van Wegen, E.E., Ket, J.C., Heymans, M.W., 2011. Early prediction of outcome of activities of daily living after stroke: a systematic review. Stroke. 42**,** 1482-8.

Viana, R.T., Laurentino, G.E., Souza, R.J., Fonseca, J.B., Silva Filho, E.M., Dias, S.N., Teixeira-Salmela, L.F., Monte-Silva, K.K., 2014. Effects of the addition of transcranial direct current stimulation to virtual reality therapy after stroke: a pilot randomized controlled trial. NeuroRehabilitation. 34**,** 437-46.

Vines, B.W., Cerruti, C., Schlaug, G., 2008. Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. BMC Neurosci. 9**,** 103.

Ward, N.S., Newton, J.M., Swayne, O.B., Lee, L., Thompson, A.J., Greenwood, R.J., Rothwell, J.C., Frackowiak, R.S., 2006. Motor system activation after subcortical stroke depends on corticospinal system integrity. Brain. 129**,** 809-19.

Ward, N.S., Newton, J.M., Swayne, O.B., Lee, L., Frackowiak, R.S., Thompson, A.J., Greenwood, R.J., Rothwell, J.C., 2007. The relationship between brain activity and peak grip force is modulated by corticospinal system integrity after subcortical stroke. Eur J Neurosci. 25**,** 1865-73.

Wessel, M.J., Zimerman, M., Hummel, F.C., 2015. Non-invasive brain stimulation: an interventional tool for enhancing behavioral training after stroke. Front Hum Neurosci. 9**,** 265.

Wiethoff, S., Hamada, M., Rothwell, J.C., 2014. Variability in response to transcranial direct current stimulation of the motor cortex. Brain Stimul. 7**,** 468-75.

Williams, J.A., Pascual-Leone, A., Fregni, F., 2010. Interhemispheric modulation induced by cortical stimulation and motor training. Phys Ther. 90**,** 398-410.

Woods, D.L., Wyma, J.M., Yund, E.W., Herron, T.J., Reed, B., 2015. Age-related slowing of response selection and production in a visual choice reaction time task. Front Hum Neurosci. 9**,** 193.

Zheng, X., Schlaug, G., 2015. Structural white matter changes in descending motor tracts correlate with improvements in motor impairment after undergoing a treatment course of tDCS and physical therapy. Front Hum Neurosci. 9**,** 229.

Ziemann, U., Ilic, T.V., Pauli, C., Meintzschel, F., Ruge, D., 2004. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J Neurosci. 24**,** 1666-72.

Zimerman, M., Heise, K.F., Hoppe, J., Cohen, L.G., Gerloff, C., Hummel, F.C., 2012. Modulation of training by single-session transcranial direct current stimulation to the intact motor cortex enhances motor skill acquisition of the paretic hand. Stroke. 43**,** 2185-91.

Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L.G., Hummel, F.C., 2013. Neuroenhancement of the aging brain: restoring skill acquisition in old subjects. Ann Neurol. 73**,** 10-5.

Appendix A. OT AUC by sequence number

A pool of 8 sequences was used for the experiments in Chapters 4 and 5. To determine whether the sequences were of equal difficulty the OT AUC from the healthy adults was grouped by sequence number and a one-way ANOVA performed.

There was no effect of sequence number on the OT AUC ($F_{7,131}$ = 0.599, p = 0.756). Mean and standard deviation values are provided in Table A1.

Table A1 Mean and standard deviation of OT AUC for each sequence

Sequence Number	n	Mean	SD
1	14	14.3	23.2
2	16	13.9	15.43
3	19	13.0	18.0
4	13	12.7	24.3
5	20	11.7	23.1
6	19	13.7	21.4
7	17	13.7	14.1
8	14	13.2	20.5

Appendix B. OT differences between younger and older healthy adults

The results presented in Chapter 4 revealed a significant BLOCK by AGE GROUP interaction for normalised OT. The results from the *post-hoc* independent samples t-tests to test for differences between groups are shown in Table B2.

Table B2 Results of independent samples t-tests between groups of normalised OT for each block.

Block	t	df	*p*
2	1.74	31.0	0.093
3	3.02	31.0	0.005
4	3.31	31.0	0.002
5	3.44	24.8	0.002
6	3.28	31.0	0.003
7	2.97	31.0	0.006
8	2.59	31.0	0.015
9	3.05	24.6	0.005
10	3.18	22.5	0.004
11	3.40	24.0	0.002
12	3.51	31.0	0.001
13	2.99	22.5	0.007

Appendix C. Baseline and change in iSP duration

The studies in Chapters 4 and 5 assessed the change in iSP duration for each tDCS condition. Tables C1 and C2 provide details of the mean (SEM) baseline iSP duration for each condition and the change post-stimulation relative to pre-stimulation.

Table C1. Baseline and change in iSP duration for healthy young and older adults.

	Left FDI				**Right FDI**			
	S	A	C	B	S	A	C	B
Younger adults								
Baseline (ms)								
Mean	24.5	23.0	24.7	22.3	23.4	24.7	24.3	22.2
(SEM)	(1.0)	(1.7)	(1.9)	(1.3)	(0.9)	(0.9)	(0.9)	(1.0)
Change (ms)								
Mean	-1.5	0.4	-1.3	1.1	1.5	0.1	0.2	4.0*
(SEM)	(0.6)	(1.3)	(1.0)	(0.9)	(0.7)	(0.9)	(1.0)	(0.6)
Older Adults								
Baseline (ms)								
Mean	26.6	28.0	29.5	29.0	28.0	27.4	26.5	27.3
(SEM)	(2.0)	(2.1)	(2.1)	(2.4)	(2.4)	(2.2)	(2.6)	(2.6)
Change (ms)								
Mean	1.8	-0.6	-0.8	-1.1	-0.3	0.4	0.9	0.3
(SEM)	(1.2)	(1.3)	(1.4)	(1.4)	(1.0)	(1.0)	(0.7)	(1.2)

FDI = first dorsal interosseous. S = sham, A = anodal, C = cathodal, B = bihemispheric. * significant difference from sham, $p < 0.05$.

Table C2. Baseline and change in iSP duration for stroke survivors.

	Ipsilesional to contralesional M1 TCI (unaffected FDI, n = 21)				**Contralesional to ipsilesional M1 TCI (affected FDI, n = 11)**			
	S	A	C	B	S	A	C	B
Baseline (ms)								
Mean	23.4	24.0	26.4	25.6	44.0	40.9	41.6	40.1
(SEM)	(2.4)	(2.4)	(2.7)	(3.0)	(6.5)	(5.6)	(5.8)	(6.0)
Change (ms)								
Mean	2.6	1.7	-0.3	0.5	-1.8	1.0	-0.6	1.6
(SEM)	(1.6)	(1.0)	(1.0)	(0.9)	(2.9)	(2.2)	(3.4)	(2.6)

FDI = first dorsal interosseous. S = sham, A = anodal, C = cathodal, B = bihemispheric.

Appendix D. Correlations between learning, function and TCI

In Chapters 4 and 5 potential relationships between changes in TCI duration and changes in motor sequence learning and JTT performance relative to sham were explored. Tables D1 and D2 present the results of the Pearson correlations for healthy adults (Chapter 4) and stroke survivors (Chapter 5), respectively.

Table D1 Pearson correlation results for healthy adults.

TCI change:	**Left to right M1**		**Right to left M1**	
	R	*p*	*R*	*p*
OT AUC (% sham)				
Anodal	-0.102	0.586	-0.149	0.424
Cathodal	-0.159	0.393	0.103	0.580
Bihemispheric	0.057	0.762	-0.151	0.417
PI AUC (% sham)				
Anodal	-0.275	0.134	-0.019	0.919
Cathodal	-0.322	0.078	-0.290	0.114
Bihemispheric	-0.199	0.284	-0.006	0.976

OT = onset time, PI = performance index, AUC = area under the curve.

Table D2 Pearson correlation results for stroke survivors.

TCI change:	**Ipsilesional to contralesional M1**	
	R	*p*
OT AUC (% sham)		
Anodal	-0.097	0.675
Cathodal	0.031	0.895
Bihemispheric	-0.065	0.779
OT difference last block to random block (-sham)		
Anodal	-0.392	0.079
Cathodal	-0.209	0.362
Bihemispheric	-0.036	0.879
JTT % change (- sham)		
Anodal	0.235	0.305
Cathodal	0.034	0.884
Bihemispheric	-0.078	0.738

OT = onset time, AUC = area under the curve, JTT = Jebsen Taylor Test.

Appendix E. Changes in MEP amplitude with tDCS

To investigate whether the expected changes in corticospinal excitability were occurring following tDCS in Chapters 4 and 5, MEP amplitude was measured from the data collected for TCI analysis using Signal 4.07 (CED, UK). Briefly, single pulse stimuli were delivered to each M1 at 80 % MSO during active contraction of the ipsilateral FDI. Peak-to-peak MEP amplitude (mV) was determined for the contralateral FDI for each of the 20 MEPs. Any traces showing voluntary EMG activation of the contralateral FDI in the 500 ms preceding the stimulus were removed from analysis and a trimmed mean MEP amplitude (Fleming et al., 2012) determined. One-tailed paired t-tests were used to compare the change in MEP amplitude under each tDCS condition with sham.

Chapter 4 – healthy adults

For younger adults, there was a significant increase in MEP amplitude from the right M1 with cathodal tDCS compared to sham ($p = 0.012$) and a tendency for anodal ($p = 0.053$) and bihemispheric ($p = 0.064$). There were no significant changes from the left M1 ($p > 0.1$; see Figure E1). For older adults, there were no significant changes for either hemisphere ($p > 0.1$; see Figure E2).

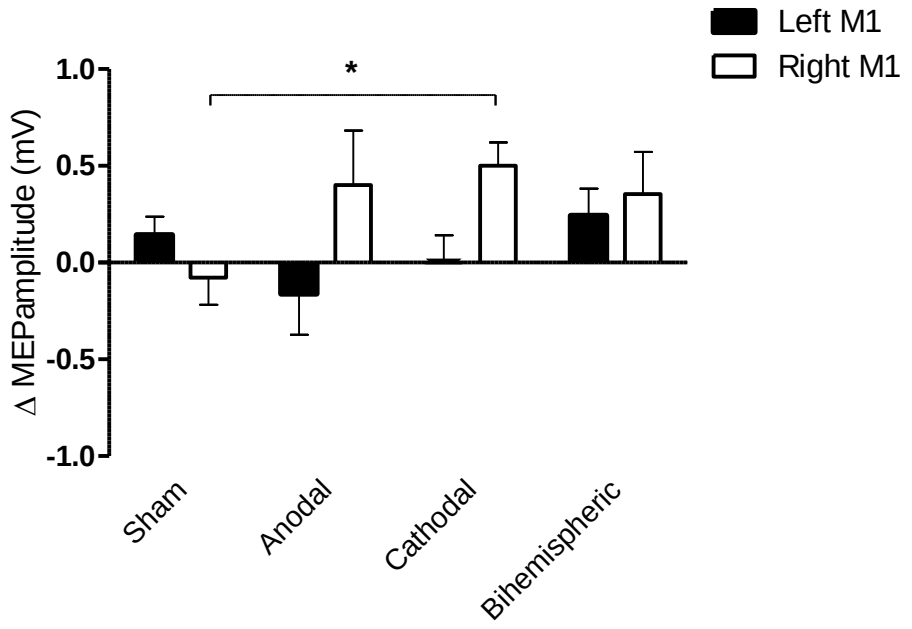


Figure E1 Change in FDI MEP amplitude under each tDCS condition for younger adults.
*significant increase in MEP amplitude from the right M1 after cathodal tDCS compared with sham ($p = 0.012$).

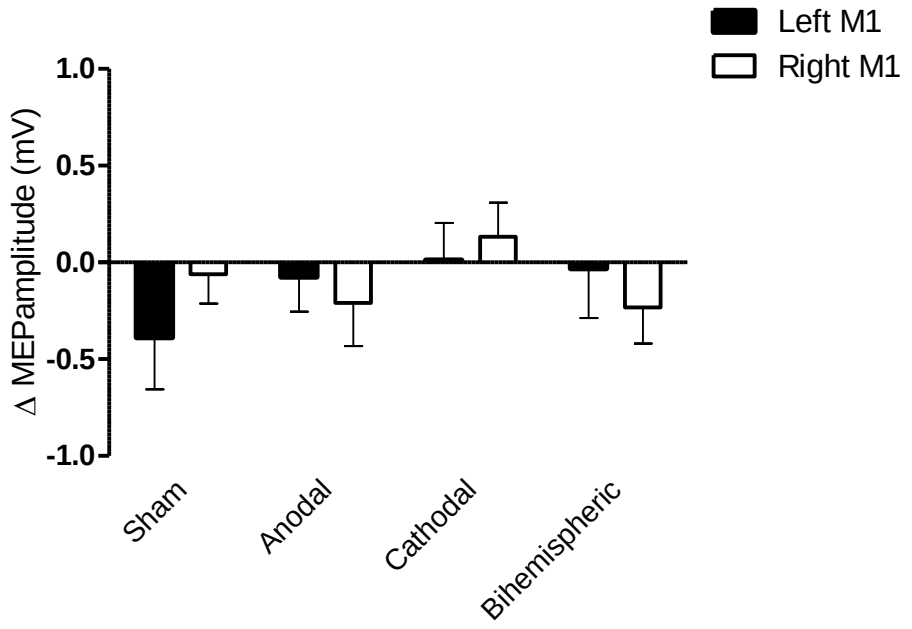


Figure E2 Change in FDI MEP amplitude under each tDCS condition for older adults.
There were no changes in MEP amplitude ($p > 0.1$).

There was a significant increase in MEP amplitude from the ipsilesional M1 with anodal tDCS compared to sham (p = 0.04), but no change for cathodal (p = 0.16) or bihemispheric (p = 0.24). For the contralesional M1 there was a significant reduction in MEP amplitude following bihemispheric tDCS (p = 0.003), but no significant change for anodal (p = 0.08) or cathodal (p = 0.10). See Figure E3.

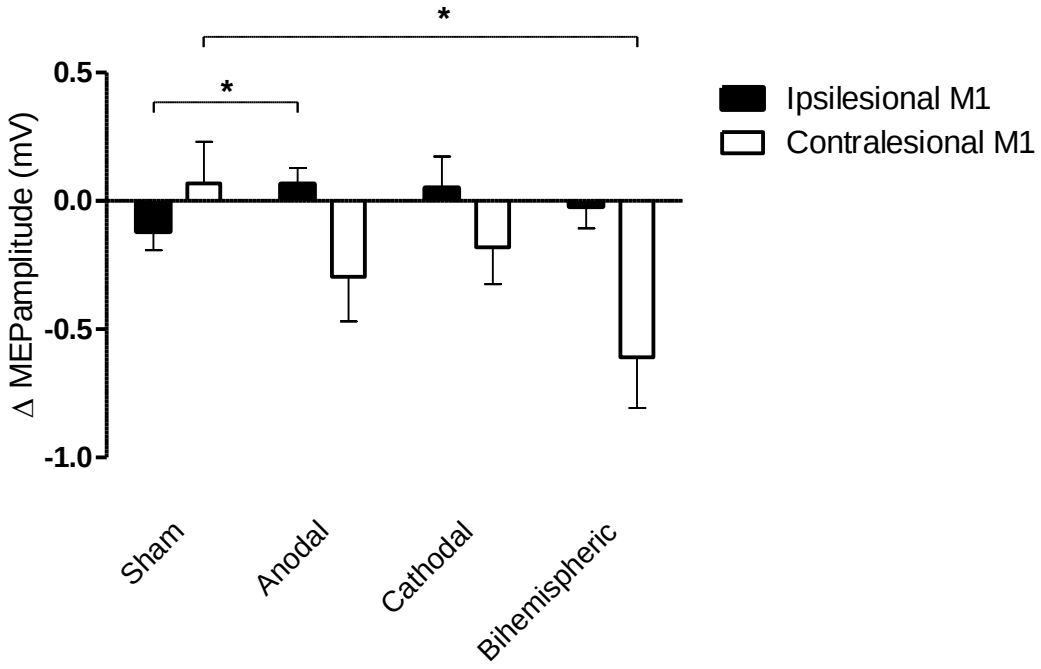


Figure E3 Change in FDI MEP amplitude (mean ± SEM) under each tDCS condition.
* significant difference from sham, p < 0.05.

Appendix F. Comparison of sequence specific learning between stroke survivors and age-matched controls

Experiment 3 of Chapter 3 demonstrated impairment in sequence specific learning for stroke survivors in comparison with age-matched healthy adults. To confirm this finding, and to assess whether tDCS impacted on this impairment, data from Chapters 4 and 5 were compared. A 4 TDCS by 2 GROUP mixed rmANOVA was used to compare the normalised OT difference between the last block of the repeated sequence and the random block across the four tDCS conditions (sham, anodal, cathodal, bihemispheric) for the stroke group and age-matched healthy adults. Average (SD) age was 59 (13) years for the stroke group and 63 (14) years for the healthy control group.

There was a significant effect of GROUP ($F_{1,41}$ = 13.353, p =0.001), but no effect of TDCS ($F_{3,123}$ = 0.151, p = 0.929) or interaction ($F_{3,123}$ = 0.879, p = 0.454). This indicates that sequence specific learning was impaired for the stroke group, regardless of tDCS condition and that tDCS did not improve the OT difference for either group (Figure F1).

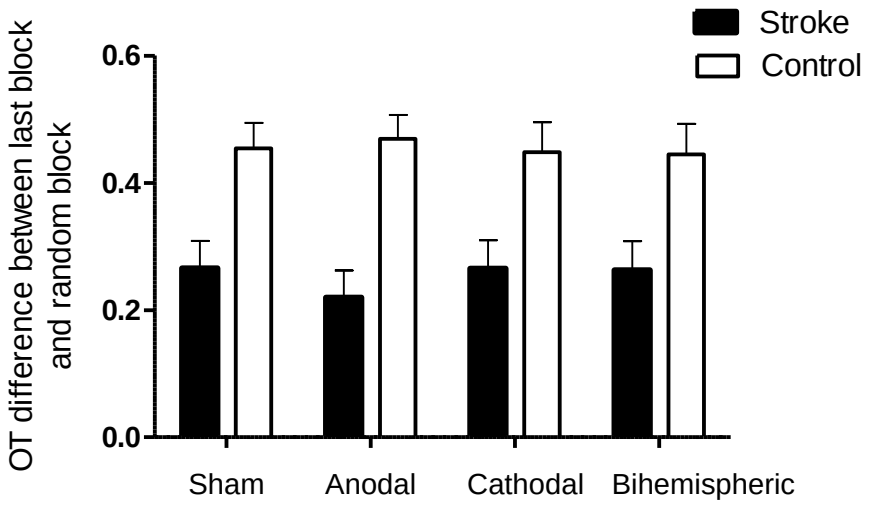


Figure F1 Normalised OT difference for stroke and control groups under each tDCS condition. There was a significant effect of group as stroke survivors showed impaired sequence specific learning across all tDCS conditions (p = 0.001).